(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 May 2001 (03.05.2001)

PCT

(10) International Publication Number
WO 01/30808 A1

(51) International Patent Classification[7]: C07K 7/00

(21) International Application Number: PCT/US99/25201

(22) International Filing Date: 27 October 1999 (27.10.1999)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant *(for all designated States except US)*: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 12th Floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MILLHAUSER, Glenn, L.** [US/US]; 111 Crestview Terrace, Santa Cruz, CA 95060 (US). **BOLIN, Kimberly, A.** [US/US]; 142 Kennan Street, Santa Cruz, CA 95060 (US). **ANDERSON, D., Joe** [US/US]; 3406 Woodland Street, Ames, IA 50014 (US).

(74) Agents: **EBERLE, Shelley, P.**; Cooley Godward LLP, 3000 El Camino Real, Five Palo Alto Square, Palo Alto, CA 94306-2155 et al. (US).

(81) Designated States *(national)*: CA, JP, US.

Published:
With international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/30808 A1

(54) Title: METHODS AND COMPOUNDS FOR MODULATING MELANOCORTIN RECEPTOR-LIGAND BINDING

(57) Abstract: The present invention relates to methods and agonist/antagonist compounds for modulating melanocortin receptor-ligand binding. The invention includes a method for identifying re[illegible]ues comprising a ligand binding site for a melanocortin receptor of interest. Also included is a method of identifying agonists and/or antagonists that bind to a ligand binding site for a melanocortin receptor of interest. Agonists and antagonists of ligand binding to melanocortin receptors also are provided. The invention is exemplified by identification and manipulation of the C-terminus of the human agouti related protein, which binds melanocortin receptors 3 and 4. The methods can be applied to other melanocortin receptor agonists and antagonists.

METHODS AND COMPOUNDS FOR MODULATING MELANOCORTIN RECEPTOR-LIGAND BINDING

INTRODUCTION

Technical Field

The present invention relates to methods and compounds for modulating melanocortin receptor-ligand binding.

Background

Recent biochemical investigations have identified agouti related protein ("AGRP") as playing a major role in the regulation of mammalian feeding behavior. The human AGRP is a 132 residue polypeptide (SEQ ID NO:1) that is a naturally occurring competitive antagonist of melanocortin receptors 3 and 4 ("MC3r" and "MC4r"), the overexpression of which results in adult onset obesity and diabetes in mice (Shutter, et al., *Genes Dev.* 11:593-602 (1997); Huszar, et al., *Cell* 88:131-141 (1997); Hahn, et al., *Nature Neurosci.* 1:271-171 (1997)). AGRP binding to MC4r in particular is the subject of intense interest since knockout mice that do not express MC4r exhibit the same phenotype as caused by overexpression of AGRP (Huszar, et al., *supra*). There is also evidence for the parallel expression of AGRP and neuropeptide Y in the arcuate nucleus of the hypothalamus, with neuropeptide Y known to stimulate feeding (Hahn, et al., *supra*). This region of the brain also expresses MC4r and is involved in energy homeostasis. Research has also focused on other melanocortin receptors, their antagonists and methods for modulating receptor activity. See for example, Wei, et al., WO9943709.

The growing body of evidence linking AGRP to weight control has yet to elucidate its exact mechanism of action. However, studies on AGRP do benefit from analogy to the much more widely studied agouti protein, as AGRP was originally identified through the homology of its C-terminal region with the same region of the agouti protein (Shutter, et al., *supra*). The agouti protein has been a focal point in obesity research for a number of years, since ectopic expression of the wild-type protein in mice leads to obesity and related disorders, i.e. the same symptoms as the overexpression of the more recently identified AGRP (Klebig, et al., *Proc. Natl. Acad. Sci.* USA 92:4728-4732 (1995); Michaud, et al., *J. Endocrinol* 155:207-209 (1997). However, unlike AGRP, agouti has distinct expression patterns in mice and humans, making *in vivo* work with mice less applicable to human obesity disorders. AGRP, like agouti, is selective

for MC3r and MC4r but has approximately 100-fold greater binding affinity than agouti at these receptors (Fong, et al., *Biochem. Res. Commun.* 237:629 611 (1997)).

While full length agouti and AGRP are only 25% homologous, in their 46 residue Cys-rich C-terminal regions, nine of the 10 Cys residues are spatially conserved and there are a further 10 identical residues giving ~40% sequence identity. Three consecutive, conserved residues RFF (111-113 in human AGRP) were determined to be essential to the biological activity of both agouti (Kiefer, et al., *Biochemistry* 36:2084-2090 (1997); Kiefer, et al., *Biochemistry* 37:991-997 (1998)) and AGRP (Tota, et al., *Biochemistry* 38:897-904 (1999)). Two recent investigations have shown that the chemically synthesized C-terminal region of AGRP competitively antagonizes α-melanocyte stimulating hormone (α-MSH) at melanocortin receptors with equal or greater potency than the full proteins (Quillan, et al., *FEBS Lett.* 428:59-62 (1998); Yang, et al., *Mol. Endocrinol.* 13:148-155 (1999)), consistent with similar findings for agouti (Willard, et al., *Biochemistry* 34:12341-12346 (1995)). Thus the Cys-rich C-terminal region of AGRP, referred to as minimized agouti related protein ("MARP") is a prime candidate for structural studies:

$$CVRLH_5ESCLG_{10}QQVPC_{15}CDPCA_{20}TCYCR_{25}$$

$$FFNAF_{30}CYCRK_{35}LGTAM_{40}NPCSR_{45}T \text{ (SEQ ID NO:2)}$$

The covalent structure of MARP exhibits five disulfide bonds, which exist between the following ten Cys residues (Bures, et al., *Biochemistry* 37:12172-12177 (1998)): Cys_1 and Cys_{16}; Cys_8 and Cys_{22}; Cys_{15} and Cys_{33}; Cys_{19} and Cys_{43}; Cys_{24} and Cys_{31}.

Despite the important biological activities of AGRP, no experimental 3D structure has been available for this protein. The inhibitor cystine knot ("ICK") family of proteins are also disulfide-rich and the structures of these invertebrate toxins have been used to suggest possible structures for the agouti and AGRP C-terminal regions (Kiefer, et al., *Biochemistry* 37:991-997 (1998); Tota, et al., *supra*). Indeed, the recently reported disulfide map for AGRP and a construct containing the C-terminal domain demonstrate ICK-like pairings (Norton, et al., *Toxicon* 36:1573-1583 (1998)) of the 10 Cys residues: 1-16, 8-22, 15-33, 19-43, 24-31 (using MARP numbering, Bures, et al., *supra*). Beyond such modeling, the only structural data published on either agouti or AGRP are circular dichroism (CD) spectra which suggest that both proteins have little regular secondary structure, although there may be some β-sheet structure, consistent with ICK structural characteristics (Willard, et al., *supra*; Rosenfeld, et al., *Biochemistry* 37:16041-16052 (1998)).

Accordingly, there exists a need for a better understanding of the structure of AGRP and related proteins such as MARP. More particularly, a need exists for the identification and characterization of the receptor binding regions of AGRP, MARP and other ligands for melanocortin receptors such as MC3r and MC4r, and molecules that affect their interaction with agouti and AGRP proteins. This would provide a major new target for iterative drug design, synthesis, and selection. It also would be advantageous to devise methods and compositions for reducing the time required to discover compounds that target the ligand binding site of melanocortin receptors and administer them to organisms, and mammals in particular, to modulate physiological processes regulated by melanocortin receptors.

The instant invention meets that need in describing the 3D structure in solution of the human AGRP Cys-rich C-terminal region.

SUMMARY OF THE INVENTION

The present invention relates to the receptor binding region of the human agouti related protein ("AGRP"), which binds to melanocortin receptors 3 and 4 ("MC3r" and "MC4r"). Information about this region permits design of compounds that bind to the ligand binding site of melanocortin receptors and modulate ligand binding to the receptor. The compounds include agonists and antagonists that modulate melanocortin receptor activity by promoting (agonists) or blocking (antagonists) ligand binding to the receptor, particularly antagonists. The compounds of the invention can be receptor-, cell- and/or tissue-specific.

The present invention also includes an NMR structure of the human AGRP C-terminus ("MARP"), which binds to the ligand binding site of melanocortin receptors. The NMR structure provides a means to obtain atomic modeling information of the specific amino acids and their atoms forming the receptor binding region and that interact with molecules located at the ligand binding site.

The present invention further provides methods for identifying and designing small molecules that bind to the ligand binding site using atomic models of MARP. The method involves modeling test compounds that mimic or match the 3D conformation of MARP and therefore are expected to fit spatially into a melanocortin receptor ligand binding site of interest, using an atomic structural model comprising the MARP receptor binding region or portion thereof, screening the test compounds in a biological assay characterized by binding of a test compound to a melanocortin receptor ligand binding site, and identifying a test compound that modulates ligand binding to the melanocortin receptor.

The invention also includes compositions and methods for identifying ligand binding sites of melanocortin receptors. The methods involve examining the MARP surface to identify residues that modulate ligand binding. The residues can be identified by homology to the receptor binding region of MARP, as described herein. Overlays and superpositioning with a three dimensional model of the MARP receptor binding region, or a portion thereof that contains a receptor binding region, also can be used for this purpose. Additionally, alignment and/or modeling can be used as a guide for the placement of mutations on the receptor binding region surface to characterize the nature of the ligand binding site on melanocortin receptors in the context of a cell.

Also provided is a method of modulating the activity of a melanocortin receptor. The method can be *in vitro* or *in vivo*. The method comprises administering, *in vitro* or *in vivo*, a sufficient amount of a compound that binds to the ligand binding site. Preferred compounds bind to the site with greater affinity than ligand proteins found in a cell of interest.

The invention further includes a method for identifying an agonist or antagonist of ligand binding to a melanocortin receptor. The method comprises providing the atomic coordinates comprising a melanocortin receptor binding region or portion thereof to a computerized modeling system; modeling compounds which match or mimic the receptor binding region and thus fit spatially into the melanocortin receptor ligand binding site; and identifying in an assay for melanocortin receptor activity a compound that increases or decreases activity of the melanocortin receptor through binding the ligand binding site. The melanocortin receptor binding region is preferably the MARP receptor binding region or portion thereof.

Also provided is a machine-readable data storage medium with information for constructing and manipulating an atomic model comprising a receptor binding region or portion thereof. The medium comprises a data storage material encoded with machine readable data which, when using a machine programmed with instructions for using said data, is capable of displaying a graphical three-dimensional representation of a molecule that binds a melanocortin receptor.

Also provided is a method of identifying a compound that selectively modulates the activity of one type of melanocortin receptor compared to other melanocortin receptors. The method is exemplified by modeling test compounds that fit spatially and preferentially into a melanocortin receptor ligand binding site of interest using an atomic structural model of the MARP receptor binding region, selecting a compound that mimics or matches one or more residues of the receptor binding region unique in the context of that region, and identifying in an

assay for ligand binding activity a compound that selectively binds to the ligand binding site compared to other melanocortin receptors.

The invention finds use in the selection and characterization of peptide, peptidomimetic, as well as other small molecule compounds, such as small organic molecules, identified by the methods of the invention, particularly new lead compounds useful in treating melanocortin receptor-based disorders. The invention also includes a group of polypeptides or peptide-related compounds comprised of sequences from MARP. Members of this group may be made synthetically and can be modified in myriad ways.

The invention includes a method of treating a disease state in mammals by treatment with a polypeptide comprised of sequences from MARP. A preferred embodiment would be a method of treating a wasting syndrome, such as HIV wasting syndrome, cachexia, or anorexia.

The invention further includes a method of screening for a compound that is capable of inhibiting binding of a melanocortin agonist or antagonist to a melanocortin receptor. This method can be used to screen for compounds that inhibit binding at specific melanocortin receptors such as MC3R or MC4R. Further, this method can be used to identify compounds that interfere with either agonist or antagonist binding at the melanocortin receptor. Examples of antagonists that interfere with agonist binding include, but are not limited to, MARP and AGRP.

The invention also includes methods of screening for binding of compound to a melanocortin receptor. Such methods can be used to screen for compounds that bind specific melanocortin receptors such as MC3R and MC4R, and can be used for high throughput screening.

The invention also pertains to a polypeptide molecule that acts as a strong antagonist of melanocortin receptor types 3 and 4, and is useful for the treatment of eating disorders and obesity.

DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the C_α backbone of the MARP minimized average structure. The N-terminal loop, central loop and C-terminal loop are indicated. Disulfide bonds are represented by dashed lines. Spheres represent residues with amides protected from HX for more than 12 hours, more than 24 hours and more than 8 days.

Fig. 2 depicts the heavy atom (non-hydrogen) backbone representation of MARP for 14 NMR structures with residues 1-34 fit to the minimized average structure (Root Mean Square Deviation, "RMSD" 1.49Å). Only the minimized average structure (thick cylinder) is shown for the more disordered C-terminal loop.

Fig. 3 shows the backbone atoms for residues 24-31 of the family of 20 structures with residues 24-31 fit to the minimized average structure. The side chain heavy atoms of residues 25, 26 and 27, essential for activity, are shown.

DESCRIPTION OF SPECIFIC EMBODIMENTS

The agouti related protein ("AGRP") is a mammalian signaling molecule, involved in weight homeostasis, that causes adult onset obesity when overexpressed in mice. AGRP was originally identified by homology to the agouti protein, another potent signaling molecule involved in obesity disorders in mice. While AGRP's exact mechanism of action is unknown, it has been identified as a competitive antagonist of melanocortin receptors 3 and 4 ("MC3r" and "MC4r"). MC4r in particular is implicated in the hypothalamic control of feeding behavior. Full length agouti and AGRP are only 25% homologous, however, their active C-terminal regions are ~40% homologous, with nine out of the 10 Cys residues spatially conserved. Until now, 3D structures have not been available for either agouti, AGRP or their C-terminal regions.

This invention relates to the 3D NMR structure in solution of the human AGRP Cys-rich C-terminal region as determined by ^{1}H NMR using a protein prepared by total chemical synthesis. As used herein the term "the NMR structure" is understood to refer to the minimized average of the family of NMR structures. Because biochemical investigations demonstrate that this minimal region retains full biological activity, this protein is referred to herein as minimized agouti related protein ("MARP"). MARP residues 1-46 (SEQ ID NO:2), correspond to human AGRP residues 87-132 (residues 87-132 of SEQ ID NO:1). Thus, human AGRP numbering is obtained by adding 86 to MARP numbering.

MARP's topology is characterized by three large loops (referred to herein as the N-terminal loop, the central loop and the C-terminal loop), with four of the five disulfide bridges at the base of the structure, and an absence of canonical secondary structure such as helices or sheets. Two of the three loops are structurally well characterized by the NMR data as indicated by low RMSDs. The region of MARP containing the RFF triplet (Tota, et al., *supra*) (residues 25-27 in MARP) necessary for function is located in one of the best defined regions of the protein. While previously reported structural models of the C-terminal region of AGRP were attempted based on Cys homology between AGRP and certain toxin proteins, Cys spacing is not sufficient to correctly determine the 3D fold of the molecule. Despite the conservation of Cys spacing and the disulfide map between MARP and other small disulfide-rich proteins from the

ICK family, it is apparent from the structure described herein that MARP does not adopt an ICK-like fold.

The 3D structure of MARP presents a basis for the development of methods and compositions for identifying compounds that modulate melanocortin receptor activity, in particular the activity of MC3r and MC4r. In a preferred embodiment, the 3D structure presents a basis for compounds that preferentially modulate MC4r activity. The compounds can be melanocortin receptor agonists or antagonists that bind to the ligand binding site (and that act as mimetics to the ligand in this regard), and promote (agonists) or block (antagonists) binding of the ligand to the target melanocortin receptor. Compounds that bind to the ligand binding site also are provided. The compounds can be natural or synthetic. Preferred compounds are small organic molecules, peptides and peptidomimetics (e.g., cyclic peptides, peptide analogs, or constrained peptides).

Accordingly, one aspect of the invention involves methods for identifying and designing small molecules that bind to the ligand binding site using atomic models of MARP. In particular, the invention provides for a method of identifying a compound that modulates ligand binding to a melanocortin receptor. The method involves modeling test compounds that mimic or match the 3D conformation of MARP and therefore are expected to fit spatially into a melanocortin receptor ligand binding site of interest, using an atomic structural model of a melanocortin receptor binding region or portion thereof, preferably comprising the MARP receptor binding region or portion thereof. The test compounds can fit spatially into the ligand binding site of interest based upon a geometric fit of its three-dimensional structure or based upon the spatial arrangement of atoms presenting specific chemical properties such as charge and hydrophobicity. The test compounds are then screened in an assay, such as a biological assay, characterized by binding of a test compound to a melanocortin receptor ligand binding site, and identifying a test compound that modulates ligand binding to the melanocortin receptor. Details of the atomic structural model are described in detail below.

As noted above, the 3D structure of MARP is characterized by three loops held together at the base by an apparent scaffold of four disulfide bonds 1-16, 8-22, 15-33 and 19-43. The fifth disulfide bond, 24-31, further stabilizes the base of the active loop which presents the RFF triplet on the protein surface. There is no identifiable canonical helical or sheet structure. It is clear from biochemical data that the RFF triplet is critical for the activity of MARP as a competitive antagonist of α MSH stimulated activation of MC4r signaling. The structure described herein shows that MARP is structured to present the side chains of the RFF triplet on

the surface of the protein and to the surrounding solvent. Recent work demonstrates that MARP is much more active than smaller AGRP derived peptides containing the RFF triplet (Tota, et al., *Biochemistry* 38:897-904 (1999)). Thus, the detailed fold of the central loop and perhaps the presence of the N- and C-terminal loops are critical for AGRP function. In addition, based upon work with chimeras of melanocortin receptors (I. Gantz, unpublished data), it is possible that the N- and C-terminal loops may confer receptor subtype specificity.

The previous absence of structural data on both AGRP and agouti encouraged the modeling of the C-terminal regions of these proteins onto the ICK family (Norton, et al., *supra*)) which is characterized by homologous Cys spacing (Kiefer, et al., *Biochemistry* 37:991-997 (1998); Tota, et al., *supra*). The ICK family of proteins primarily consists of small (<60 residues) disulfide-rich (three or four disulfides) toxin proteins from the venom of spiders and cone snails, which function as ion channel antagonists (Norton, et al., *supra*). The coincidence between the function of the majority of these toxins and the recent description of part of the agouti protein's mechanism of action being calcium dependent (Kim, et al., *FASEB J.* 10:1646-1652 (1996); Kim, et al., *Am. J. Physiol.* 272:E379-384 (1997); Jones, et al., *Am. J. Physiol.* 270:E192-196 (1996)) further encouraged these homology modeling efforts. The ICK motif in particular is characterized by the topology of the three disulfide bonds corresponding to 1-16, 8-22 and 15-33 in MARP. In the ICK motif the first two disulfide bonds with their intervening main chain atoms form a topological circle through which the third disulfide bond passes, forming the *cystine knot* (Norton, et al., *supra*). The motif is further characterized by the identification of an irregular triple stranded antiparallel β-sheet, roughly corresponding to residues 6-8, 20-24 and 31-34 in MARP. The remaining two disulfide bonds in MARP each occur in individual ICK proteins as separate examples of potential "non-motif" disulfide bonds, although no examples of ICK motif proteins with five disulfide bonds have been observed.

Despite these apparent similarities, the experimental structure of MARP shows that this protein does not satisfy the criteria required for inclusion in the ICK family. While the first two disulfide bonds in MARP 1-16 and 8-22, together with the polypeptide backbone form a topological circle, none of the remaining disulfides passes through the circle to form a cystine knot. Instead, disulfide bond 15-33 is positioned adjacent to the circle with all of the fold on one side of this circle. In addition, MARP lacks the β-sheet found in ICK family proteins. The experimental determination of the distinctive 3D structure of MARP described herein suggests that Cys spacing and even the disulfide map of small Cys-rich proteins may not always be

sufficient to accurately predict protein folds. These results speak to the potential limitations of "homology modeling" of protein structures, and may have important implications for the emerging field of genomic structural biology. Use of atomic modeling, alone or in combination with homology modeling may serve to remedy this.

As described in the Examples, ligand binding studies, and analysis of atomic models derived from the MARP NMR structure reveal for the first time a previously unknown structure for MARP and its receptor binding region. By "receptor binding region" is intended a structural segment or segments of melanocortin receptor ligands, and MARP in particular, folded in such a way so as to give the proper geometry and amino acid residue conformation for binding to a melanocortin receptor. By "ligand binding site" is intended a structural segment or segments of melanocortin receptor polypeptide chain folded in such a way so as to give the proper geometry and amino acid residue conformation for binding a ligand. These are the physical arrangement of protein atoms in three-dimensional space forming a receptor binding region or a ligand binding site pocket or cavity.

The MARP structure has three major loops: the N-terminal loop, residues 1-18 (residues 1-18 of SEQ ID NO:2), the central loop, residues 19-34 (residues 19-34 of SEQ ID NO:2) and the C-terminal loop, residues 35-46 (residues 35-46 of SEQ ID NO:2). Residues forming the receptor binding region are amino acids corresponding to (i.e., the same as or equivalent to) residues 24-31 of the central loop (residues 24-31 of SEQ ID NO:2), referred to herein as the "active" loop. In particular, residues 25, 26 and 27 (the "RFF" triplet) of the active loop are critical for activity. It has been found that a polypeptide comprising the central loop, preferably at least a portion of the N-terminal loop, is desirable for optimal biological activity. As used herein, the term "at least a portion of the N-terminal loop" is intended to mean a sequence that corresponds to (i.e., the same as or equivalent to), at least residues 15 to 18 of the N-terminal loop (residues 15-18 of SEQ ID NO:2), preferably at least residues 8 to 18 of the N-terminal loop (residues 8-18 of SEQ ID NO:2), and is also intended to include all of the N-terminal loop, i.e., residues 1 to 18 of the N-terminal loop (residues 1-18 of SEQ ID NO:2). The term is also intended to mean a sequence that corresponds to at least 20%, preferably at least 60%, and more preferably at least 90%, of the contiguous or non-contiguous amino acid residues or their atoms selected from amino acid residues 1 to 18 of the N-terminal loop (residues 1-18 of SEQ ID NO:2).

The invention also includes compositions and methods for identifying receptor binding regions of melanocortin receptor agonist and antagonists, along with ligand binding sites of

melanocortin receptors. The methods involve examining the surface of a polypeptide of interest to identify residues that modulate ligand binding. The residues can be identified by homology to the receptor binding region of MARP described herein. A preferred method is alignment with the residues of any polypeptide corresponding to (i.e., the same as or equivalent to) residues 1-18 of the N-terminal loop (residues 1-18 of SEQ ID NO:2), residues 19-34 of the central loop (residues 19-34 of SEQ ID NO:2) and residues 35-46 of the C-terminal loop (residues 35-46 of SEQ ID NO:2). Overlays and superpositioning with a three-dimensional model of the MARP receptor binding region, or a portion thereof that contains a receptor binding region, also can be used for this purpose. For example, melanocortin receptor agonists and antagonists identifiable by homology alignment include naturally occurring compounds or compounds structurally related to such naturally occurring compounds found in humans, along with synthetic compounds.

Alignment and/or modeling also can be used as a guide for the placement of mutations on the receptor binding region surface to characterize the nature of the ligand binding site on melanocortin receptors in the context of a cell. To destroy the ligand binding interaction, preferred mutations are to charged residues (e.g., Arg, Lys, or Glu) on the basis that bulky, surface charged residues might disrupt ligand binding, yet preserve the overall ligand structure and solubility. Mutants can be tested for ligand binding as well as the relative change in strength of the binding interaction. Ligand-dependent ligand interaction assays also can be tested for this purpose, such as those described herein.

Compounds that bind to the ligand binding site of melanocortin receptors can be identified by computational modeling and/or screening. For example, ligand agonists or antagonists can be identified by providing atomic coordinates comprising the MARP receptor binding region or portion thereof to a computerized modeling system, modeling them, and identifying compounds that mimic or match the receptor binding region and thus would be expected to fit spatially into the ligand binding site. By a "portion thereof" is intended the atomic coordinates corresponding to a sufficient number of residues or their atoms of the receptor binding region that interact with a melanocortin receptor capable of binding the region. As another example, an atomic structural model utilized for computational modeling and/or screening of compounds that mimic or match the receptor binding region and thus would be expected to fit spatially into the ligand binding site, may include a portion of atomic coordinates of amino acid residues corresponding to the region composed of residues 24-31 of the central loop (residues 24-31 of SEQ ID NO:2), or their structural and functional equivalents. An atomic

model can also be designed that includes residues 19-34 of the central loop (residues 19-34 of SEQ ID NO:2), and a model can further be designed that includes residues 19-34 of the central loop (residues 19-34 of SEQ ID NO:2) and some or all of residues 1-18 of the N-terminal loop (residues 1-18 of SEQ ID NO:2). Thus, for example, the atomic coordinates provided to the modeling system can contain atoms of MARP, all or part of the receptor binding region or a subset of atoms useful in the modeling and design of compounds that mimic or match the receptor binding region.

The atomic coordinates of MARP and its receptor binding region, can be used for modeling to identify other compounds or fragments that bind melanocortin receptors. By "modeling" is intended quantitative and qualitative analysis of molecular structure/function based on atomic structural information and receptor-ligand agonists/antagonists interaction models. This includes conventional numeric-based molecular dynamic and energy minimization models, interactive computer graphic models, modified molecular mechanics models, distance geometry and other structure-based constraint models. Modeling is preferably performed using a computer and may be further optimized using known methods. By "fits spatially" is intended that the three-dimensional structure of a compound is accommodated geometrically by a cavity or pocket of a melanocortin receptor ligand binding site or by a pattern of charge or hydrophobicity.

Compounds of particular interest fit spatially and preferentially into the ligand binding site. By "fits spatially and preferentially" is intended that a compound possesses a three-dimensional structure and conformation for selectively interacting with a melanocortin receptor ligand binding site. Compounds that fit spatially and preferentially into the ligand binding site interact with amino acid residues forming the ligand binding site. More specifically, these compounds mimic or match the MARP receptor binding region. The present invention also includes a method for identifying a compound capable of selectively modulating ligand binding to different melanocortin receptors. The method comprises the steps of modeling test compounds that fit spatially and preferentially into the ligand binding site of a melanocortin receptor of interest using an atomic structural model of a MARP, screening the test compounds in a biological assay for melanocortin receptor activity characterized by preferential binding of a test compound to the ligand binding site of a melanocortin receptor, and identifying a test compound that selectively modulates the activity of a melanocortin receptor. Such receptor-specific compounds are selected that exploit differences between the ligand binding sites of one type of receptor versus a second type of receptor.

The invention also is applicable to generating new compounds that distinguish melanocortin receptor isoforms. This can facilitate generation of either tissue-specific or function-specific compounds.

The receptor-specific compounds of the invention preferably interact with conformationally constrained residues of the ligand binding site that are conserved among one type of receptor compared to a second type of receptor. "Conformationally constrained" is intended to refer to the three-dimensional structure of a chemical or moiety thereof having certain rotations about its bonds fixed by various local geometric and physical-chemical constraints. Conformationally constrained structural features of a ligand binding site include residues that have their natural flexible conformations fixed by various geometric and physical-chemical constraints, such as local backbone, local side chain, and topological constraints. These types of constraints are exploited to restrict positioning of atoms involved in receptor-ligand recognition and binding.

As described in the Examples, residues 25, 26 and 27 of the MARP receptor binding region are shown to be critical for activity. These three residues, along with the overall structure and composition of the N-terminus and central loop, appear to be necessary for optimal biological activity. Accordingly, modification to these residues can be exploited in the identification and design of compounds that modulate ligand binding to one melanocortin receptor compared to another.

For modeling, docking algorithms and computer programs that employ them can be used to identify compounds that match or mimic the MARP receptor binding region. For example, docking programs can be used to predict how a small molecule of interest can interact with the melanocortin receptor ligand binding site. Fragment-based docking also can be used in building molecules *de novo* inside the ligand binding site, by placing chemical fragments that complement the site to optimize intermolecular interactions. The techniques can be used to optimize the geometry of the binding interactions. This design approach has been made possible by identification of the receptor binding region structure thus, the principles of molecular recognition can now be used to design a compound which matches the structure of this region. Compounds that structurally match or mimic the MARP receptor binding region serve as a starting point for an iterative design, synthesis and test cycle in which new compounds are selected and optimized for desired properties including affinity, efficacy, and selectivity. For example, the compounds can be subjected to addition modification, such as replacement and/or addition of R-group substituents of a core structure identified for a particular class of binding

compounds, modeling and/or activity screening if desired, and then subjected to additional rounds of testing.

Computationally small molecule databases can be screened for chemical entities or compounds that can bind in whole, or in part, to a melanocortin receptor ligand binding site of interest. In this screening, the quality of fit of such entities or compounds to the binding site may be judged either by shape complementarity (DesJarlais et al., *J. Med. Chem.* (1988) *31*:722-729) or by estimated interaction energy (Meng et al., *J. Comp. Chem.* (1992) *13*:505-524). The molecule databases include any virtual or physical database such as electronic and physical compound library databases, and are preferably used in developing compounds that modulate ligand binding.

Compounds can be designed intelligently by exploiting available structural and functional information by gaining an understanding of the quantitative structure-activity relationship (QSAR), using that understanding to design new compound libraries, particularly focused libraries having chemical diversity of one or more particular groups of a core structure, and incorporating any structural data into that iterative design process. For example, one skilled in the art may use one of several methods to screen chemical entities or fragments to compare them to the 3D structure of the AGRP C-terminus and thus, their ability to associate with the ligand binding site of a melanocortin receptor of interest. This process may begin by visual inspection of, for example, the receptor binding region on the computer screen. Selected fragments or chemical entities may then be positioned into all or part of the region. Docking may be accomplished using software such as Quanta and Sybyl, followed by energy minimization and molecular dynamics with standard molecular mechanics force-fields, such as CHARMM and AMBER.

Residues comprising a receptor binding region can be modeled to look for energetic contributions and interaction with the bound chemical entity. For example, a compound or fragment can be designed to contain hydrophobic groups that interact with hydrophobic residues of the ligand binding site.

Specialized computer programs may also assist in the process of selecting chemical entity fragments or whole compounds. These include: GRID (Goodford, *J. Med. Chem.* (1985) *28*:849-857; available from Oxford University, Oxford, UK); MCSS (Miranker et al., *Proteins: Structure, Function and Genetics*, (1991) *11*:29-34; available from Molecular Simulations, Burlington, MA); AUTODOCK (Goodsell et al., *Proteins: Structure, Function and Genetics* (1990) *8*:195-202; available from Scripps Research Institute, La Jolla, CA); and DOCK (Kuntz

et al, *J. Mol. Biol.* (1982) *161*:269-288; available from University of California, San Francisco, CA).

Additional commercially available computer databases for small molecular compounds include Cambridge Structural Database and Fine Chemical Database (Rusinko, *Chem. Des. Auto. News* (1993) *8*:44-47).

Once suitable chemical entities or fragments have been selected, they can be assembled into a single compound. Assembly may be proceeded by visual inspection of the relationship of the fragments to each other on the three-dimensional image displayed on a computer screen in relation to the structure coordinates of a melanocortin receptor. This can be followed by manual model building using software such as Quanta or Sybyl.

Useful programs to aid one of skill in the art in connecting the individual chemical entities or fragments include: CAVEAT (Bartlett et al., "CAVEAT: A Program to Facilitate the Structure-Derived Design of Biologically Active Molecules", In: *Molecular Recognition in Chemical and Biological Problems*", Special Pub., *Royal Chem. Soc.* (1989) *78*:182-196; CAVEAT is available from the University of California, Berkeley, CA); 3D Database systems such as MACCS-3D (MDL Information Systems, San Leandro, CA; reviewed in Martin, *J. Med. Chem.* (1992) *35*:2145-2154); and HOOK (available from Molecular Simulations, Burlington, MA).

In addition to building a compound in a step-wise fashion, one fragment or chemical entity at a time as described above, compounds that bind to a ligand binding site of interest also may be designed as a whole or *de novo* using some portion(s) of the AGRP C-terminus, which is a molecule known to bind to the site. These methods include: LUDI (Bohm, *J. Comp. Aid. Molec. Design* (1992) *6*:61-78; LUDI is available from Biosym Technologies, San Diego, CA); LEGEND (Nishibata et al., *Tetrahedron* (1991) *47*:8985; LEGEND is available from Molecular Simulations, Burlington, MA); and LeapFrog (available from Tripos Associates, St. Louis, MO).

Other molecular modeling techniques may also be employed in accordance with this invention. See, for example, Cohen et al., *J. Med. Chem.* (1990) *33*:883-894); Navia et al., *Curr. Opin. Struct. Biol.* (1992) *2*:202-210). For example, where the structures of test compounds are known, a model of the test compound may be superimposed over the model of the structure of the invention. Numerous methods and techniques are known in the art for performing this step, any of which may be used. See, for example, Farmer, "*Drug Design*," Ariens, E.J., ed., 10:119-143 (Academic Press, New York, 1980); U.S. Patent No. 5,331,573; U.S. Patent No. 5,500,807; Verlinde, *Structure*, (1994) *2*:577-587); and Kuntz et al., *Science,* (1992) *257*:1078-1082). The

model building techniques and computer evaluation systems described herein are not a limitation on the present invention.

Using these computer modeling systems a large number of compounds may be quickly and easily examined and expensive and lengthy biochemical testing avoided. Moreover, the need for actual synthesis of many compounds can be substantially reduced and/or effectively eliminated.

Compounds identified through modeling can be screened in an assay characterized by binding of the compound to a ligand binding site of interest for ligand binding activity, such as a biologically based assay. Screening can be *in vitro* and/or *in vivo*. Preferred assays include cell-free competition assays and cell culture based assays. The biological screening preferably centers on activity-based response models, binding assays (which measure how well a compound binds to the receptor), and bacterial, yeast and animal cell lines (which measure the biological effect of a compound in a cell). The assays can be automated for high capacity - high throughput screening (HTS) in which large numbers of compounds can be tested to identify compounds with the desired activity.

As an example, *in vitro* binding assays can be performed in which compounds are tested for their ability to block the binding of a ligand protein, fragment, fusion or peptide thereof, to a ligand binding site of interest. For cell and tissue culture assays, they may be performed to assess a compound's ability to block function of cellular ligands, such as competitively inhibiting [Nle^4, D-Phe^7]α-MSH ("NDP-MSH"), as described in the examples and the references cited therein, which are incorporated by reference.

In a preferred embodiment, compounds of the invention bind to a melanocortin receptor ligand binding site with greater affinity than the cellular ligand proteins. Tissue profiling and appropriate animal models also can be used to select compounds. Different cell types and tissues also can be used for these biological screening assays. Suitable assays for such screening are described herein and in the Examples and the references cited therein, which references are incorporated herein in their entirety by reference. For example, ligands or binding fragments thereof can be expressed and/or assayed for their ability to inhibit cAMP generation in cells transfected with MC4r (see Example 1), as described in the examples and the references cited therein, which are incorporated by reference.

The compounds selected can have agonist and/or antagonistic properties. The compounds also include those that exhibit new properties with varying mixtures of agonist and antagonist activities, depending on the effects of altering ligand binding in the context of

different activities of melanocortin receptors which are mediated by proteins other than ligands, and which interact with the receptors at locations other than the ligand binding site. The compounds also include those, which through their binding to receptor locations that are conformationally sensitive to ligand binding, have allosteric effects on the receptor by stabilizing or destabilizing the ligand-bound conformation of the receptor, or by directly inducing the same, similar, or different conformational changes induced in the receptor by ligand binding.

Of particular interest is use of such compounds in a method of modulating melanocortin receptor activity in a mammal by administering to a mammal in need thereof a sufficient amount of a compound that fits spatially and preferentially into a ligand binding site of a melanocortin receptor of interest. By "modulating" is intended increasing or decreasing activity of a melanocortin receptor. For example, pre-clinical candidate compounds can be tested in appropriate animal models in order to measure efficacy, absorption, pharmacokinetics and toxicity following standard techniques known in the art. Compounds exhibiting desired properties are then tested in clinical trials for use in treatment of various melanocortin receptor-based disorders. These include feeding disorders, including wasting syndromes, obesity, and other disorders related to hypothalamic control of feeding. A wasting syndrome is an illness characterized by significant weight loss accompanied by other indicia of poor health, including poor appetite, gut disorder, or increased metabolic rate. Wasting syndromes include, but are not limited to, the wasting syndrome afflicting some patients diagnosed with Acquired Immune Deficiency Syndrome (AIDS) and various cancers. As methods of treating other symptoms of diseases such as AIDS progress, the incidence of wasting syndrome as the cause of death increases. Improved prophylaxis and treatment for HIV wasting syndrome is required (Kravick, et al., *Arch. Intern. Med.* 157:2069-2073, 1997). Anorexia and cachexia are well-known results of cancer that contribute to morbidity and mortality (Simons, et al, *Cancer* 82:553-560, 1998; Andrassy & Chwals, *Nutrition* 14:124-129, 1998). The reasons for the significant weight loss are multiple and may be directly related to the tumor, such as increased metabolic rate, but also include decreased intake due to poor appetite or gut involvement. Further, excessive leptin-like signaling may contribute to the pathogenesis of wasting illness (Schwartz, et al., *Pro. Nutr. Soc.* 56:785-791, 1997).

The invention also provides for the NMR structure of the human AGRP C-terminus, preferably embodied in a computer readable form. Synthesis of MARP and concentrated solutions adequate for NMR spectroscopy are described in the examples. After the NMR structure of MARP is determined, the structural information can be used in computational

methods to design synthetic agonist and antagonist compounds for the melanocortin receptors, and further structure-activity relationships can be determined through routine testing using the assays described herein and known in the art. It is expected that the structure coordinates of the NMR structure of MARP, as provided in Appendix 1, will be particularly useful for solving the NMR structure of other melanocortin receptor antagonists or agonists. The coordinates in Appendix 1 represent the minimized average of the coordinates of a family of NMR structures. The minimized average was determined by (1) calculating an average from approximately 20 NMR structures found to meet the experimental NMR distance restraints and (2) subjecting this calculated average to a final round of energy minimization.

One method that may be employed for this purpose is molecular replacement. In this method, the unknown NMR structure, may be determined using the structure coordinates of this invention as provided in Appendix 1. The Appendix 1 coordinates for the human AGRP C-terminus have been deposited with the Brookhaven National Laboratory Protein Data Bank, and have been assigned Brookhaven Protein Data Bank Accession Number 1qu8. This method will provide an accurate structural form for the unknown NMR structure more quickly and efficiently than attempting to determine such information *ab initio*.

One aspect of the invention is an NMR structure of the minimized agouti related protein, embodied in a computer readable media. Atomic coordinate information gleaned from the NMR structure of the invention is preferably stored and provided in the form of a machine-readable data storage medium. This medium contains information for constructing and/or manipulating an atomic model of a receptor binding region or portion thereof. For example, the machine readable data for the receptor binding region may comprise structure coordinates of amino acids corresponding to (i.e., the same as or equivalent to) residues 1-18 of the N-terminal loop, (residues 1-18 of SEQ ID NO:2), and/or residues 19-34 of the central loop (residues 19-34 of SEQ ID NO:2) and/or residues 35-46 of the C-terminal loop (residues 35-46 of SEQ ID NO:2), or a homologue of the molecule or molecular complex comprising the region. The machine readable data for the receptor binding region may comprise structure coordinates of amino acids corresponding to residues 1-18 of the N-terminal loop, (residues 1-18 of SEQ ID NO:2) and residues 19-34 of the central loop (residues 19-34 of SEQ ID NO:2). The machine readable data may also comprise residues 24-31 of the central loop (residues 24-31 of SEQ ID NO:2) and a portion of the N-terminal loop, for example residues 15 to 18 (residues 15-18 of SEQ ID NO:2), residues 7 to 18 (residues 7-18 of SEQ ID NO:2), or residues 1 to 18 (residues 1-18 of SEQ ID NO:2). The homologues comprise a receptor binding region that has a root mean square

deviation from the backbone atoms of the amino acids of not more than 2.54Å, preferably not more than 1.66 Å.

The machine-readable data storage medium can be used for interactive drug design and molecular replacement studies. For example, a data storage material is encoded with a first set of machine-readable data that can be combined with a second set of machine-readable data. For molecular replacement, the first set of data can comprise a Fourier transform of at least a portion of the structural coordinates of the AGRP C-terminus or portion thereof of interest, and the second data set comprises an X-ray diffraction pattern of a molecule such as the melanocortin receptor of interest. Using a machine programmed with instructions for using the first and second data sets a portion or all of the structure coordinates corresponding to the second data can be determined.

Protein for determination of the NMR structure and the assays described herein can be produced using synthetic or recombinant expression techniques. For example, AGRP C-terminal portion (MARP) can be readily synthesized using techniques described in Yang, et al. *supra*. In addition, high level expression of melanocortin receptors can be obtained in suitable expression hosts such as *E. coli*, yeast and other eukaryotic expression systems can also be used. The receptors can be expressed alone, as fragments of the mature or full-length sequence, or as fusions to heterologous sequences.

Initially, purification of the melanocortin receptor can be obtained by conventional techniques, such as hydrophobic interaction chromatography (HPLC), ion exchange chromatography (HPLC), and heparin affinity chromatography. To achieve higher purification of melanocortin receptors, the receptors can be ligand-shift-purified using a column that separates the receptor according to charge, such as an ion exchange or hydrophobic interaction column, and then bind the eluted receptor with a ligand, especially an agonist. The ligand induces a change in the receptor's surface charge such that when re-chromatographed on the same column, ligand-bound receptor is separated from unliganded receptor. Usually saturating concentrations of ligand are used in the column and the protein can be preincubated with the ligand prior to passing it over the column. The structural studies detailed herein indicate the general applicability of this technique for obtaining super-pure melanocortin receptors for use in assays to assess the antagonist or agonist activity of candidate compounds.

There are many uses and advantages provided by the present invention. For example, the methods and compositions described herein are useful for identifying peptides, peptidomimetics or small natural or synthetic organic molecules that modulate melanocortin receptor activity.

The compounds are useful in treating melanocortin receptor-based disorders. Methods and compositions of the invention also find use in characterizing structure/function relationships of natural and synthetic ligand compounds.

Another aspect of the invention also pertains to a polypeptide molecule that acts as a strong antagonist of melanocortin receptor types 3 and 4, and is useful for the treatment of eating disorders and obesity. As discussed above, research has demonstrated that the final 46 residues of the human AGRP sequence ("MARP"), possesses full receptor antagonist activity. The structure of MARP, solved by use of nuclear magnetic resonance and described herein, suggests that the final 13 residues of MARP are unstructured and thus may not play a role in receptor binding or antagonism. Thus, substantial further minimization of human AGRP may be achieved. Specifically, it is expected that the sequence (indicated by the single letter amino acid code):

$CVRLH_5ESCLG_{10}QQVPC_{15}CDP\underline{A}A_{20}TCYCR_{25}FFNAF_{30}CYC$ (SEQ ID NO:3)

which is designated "MARP-33" (based upon its 33 amino acid length) will fold with proper native-like disulfide bonds and possess the full biological activity of MARP. This sequence is equivalent to the first 33 amino acids of MARP but contain a Cys→Ala substitution at position 19 (indicated as "A") to avoid having a non-bridged thiol.

MARP-33 finds utility as a potential therapeutic for the treatment of eating disorders and obesity, and may be administered to a patient in need thereof, to increase eating and fat deposition. In addition, the MARP-33 sequence may be modified such that the modified MARP-33 may have therapeutic utility as a competitive inhibitor of *in vivo* human AGRP. Such modifications can include changing the sequence to increase binding affinity, to increase the level of antagonism and to increase stability of the molecule.

Accordingly, one aspect of the invention pertains to a method of treating a disease state in mammals that is alleviated by treatment with a polypeptide having an amino acid sequence:

CVRLHESCLGQQVPCCDPAATCYCRFFNAFCYC (SEQ ID NO:3)

which method comprises administering to a mammal in need of such a treatment a therapeutically effective amount of the polypeptide, which can be administered, by way of illustration and not limitation, in a liquid formulations or a solid formulations, such as in the form of a pharmaceutically acceptable salt thereof. Such a disease state can be a wasting syndrome. in addition, the invention encompasses a pharmaceutical composition comprising a therapeutically effective amount of a polypeptide of this sequence (SEQ ID NO:3), in a liquid,

solid or other suitable formulation, including pharmaceutically acceptable carriers, stabilizers, excipients and so forth, such as are well known in the state of the art.

In addition, the structure and sequence of MARP-33 is a useful tool in rational drug design for the design of peptide-based therapeutics. Further, the structure of this molecule will facilitate the development of non-peptide based therapeutics.

The following examples illustrate various aspects of this invention. These examples do not limit the scope of this invention.

EXAMPLES

Abbreviations

$^3J_{HN\alpha}$	three bond αH-NH scalar coupling constant
AGRP/ART	Agouti Related Protein/Agouti Related Transcript
Conformational shift	experimental chemical shift–random coil chemical shift
DQF-COSY	two-dimensional double-quantum filtered correlation spectroscopy
HX	hydrogen-deuterium exchange
ICK	Inhibitor Cystine Knot
MARP	Minimized human Agouti Related Protein, residues 87-132 of human AGRP
MC3r/MC4r	Melanocortin Receptor ¾
MRE	Mean Residue Ellipticity
α-MSH	α-Melanocyte Stimulating Hormone
NDP-MSH	[Nle^4, D-Phe^7]α-MSH, a superpotent melanocortin agonist
nOe	melanocortin Overhauser enhancement
NOESY	two-dimensional melanocortin Overhauser spectroscopy
TOCSY	two-dimensional total correlation spectroscopy

Methods and Materials

A. Chemical protein synthesis: N^{α}-Acetyl-MARP (SEQ ID NO:2) and N^{α}-acetyl-MARP(Arg25Ala) were synthesized, folded and purified to each give a protein containing five disulfide bonds, as reported in Yang, et al., *supra*.

B. CD experiments: Circular dichroism ("CD") spectra were recorded at 25°C on an Aviv 60DS spectropolarimeter in a rectangular 1 mm path length cuvet for concentrations up to 60 μM. For concentrations higher than this a round cell with a 0.1 mm path length was used. All CD samples were 50 mM potassium phosphate, pH 4.25. Concentration dependence was

ruled out in the range 20 μM-1 mM. Temperature dependence was determined for 5-85°C. The spectra are superimposable from 5 to 45°C.

C. NMR sample preparation: The activity of MARP used for the NMR sample and that of a single mutant were assayed by measuring the inhibition of cAMP production in the presence of NDP-MSH (Shutter, et al., *supra*; Huszar, et al., *supra*) in HEK-293 cells transfected with human MC4r. Control experiments were performed with no MARP. The NMR samples were found to be native-like with complete activity. NMR samples contained approximately 1.9 mM MARP at pH 4.2 in 50 mM KH_2PO_4 buffer in 90% H_2O/10% D_2O. Additional samples for HX experiments were prepared by lyophilization of protonated samples followed by reconstitution in 700 μl D_2O.

D. NMR experiments: ^1H 2D NMR spectra were principally acquired at 15°C on a Varian 500 Unity Plus spectrometer using inverse probes. NMR data were routinely acquired with a 6000 Hz spectral width, 4096 complex points in t_2, and 512 (TOCSY/DQF-COSY) or 700 (NOESY) increments in t_1. All spectra were processed using the MNMR package (Carlsberg Laboratory, Department of Chemistry, Denmark) and analyzed using XEASY (Bartels, et al., *J. Biomol. NMR* 5:1-10 (1995)), with chemical shifts referenced to 1.4-dioxime at 3.743 ppm. Sequential assignments of all backbone and >90% of side chain protons were accomplished using standard methods (Wüthrich, K. "NMR of Proteins and Nucleic Acids" (John Wiley and Sons, New York, 1986); Redfield, C. in "NMR of Macromolecules: A Practical Approach, pp. 71-99 (Roberts, G.K.C., Ed., IRL Press at Oxford University Press, Oxford, 1993)) for 50 ms TOCSY, 150 ms NOESY and DQF-COSY data. Additional data sets were acquired at 25°C and 30°C to resolve ambiguities. Examination of the three Pro residues identified nOes consistent only with trans-Pro. Four additional peaks were identified in the αN region of the TOCSY spectrum, however, associated spin systems could not be identified and neither could nOes to the peaks in question.

NOESY data for distance restraints were collected at 15°C using the WET sequence (Smallcombe, et al., *J. Magn. Reson. Ser. A* 117:295-303 (1995)) for water suppression, 1.6 s recycle delay and a mixing time of 80 ms in both H_2O and D_2O. $^3J_{HN\alpha}$coupling constants at 25°C were determined by both linear least squares fitting of the antiphase doublets in a DQF-COSY and also using the INFIT (Szyperski, et al., *J. Magn. Reson.* 99:552-560 (1992)) module of XEASY with 150 ms NOESY data. These methods agreed to within ±0.5 Hz for all of the

measured coupling constants. At 15°C larger intrinsic linewidths precluded accurate measurement of $^3J_{HN\alpha}$.

For amide exchange experiments, the magnet was preshimmed on a 21 residue peptide sample at pH 4 in D_2O/phosphate buffer. The first TOCSY experiment was begun 23 minutes after reconstituting the protonated sample in D_2O. Four TOCSY experiments identical to those described above, except for the number of t_1 increments, were acquired back to back at 15°C over a period of 24 hours. The first three consisted of 150 t_1 increments and the final experiment 300 increments. NOESY and DQF-COSY spectra were also acquired as described above.

E. Structure calculations: Final structure calculations included the covalent connectivity of the published disulfide map (Bures, et al., *supra*), and were based on a total of 414 interproton distance constraints derived from the 80 ms 2D NOESY spectra and 34 backbone Φ dihedral angle constraints derived from coupling constant measurements, giving a total of 448 total restraints, or 9.7 restraints per residue. The distance restraints can be broken down into 228 intraresidue (backbone to side chain only), 129 sequential, 20 medium range ($1<{}_1i\text{-}j_1\leq 5$) and 37 long range (${}_1i\text{-}j_1>5$) restraints. These restraints were assigned as strong, medium or weak. The total numbers of restraints in each category were 95 strong, 246 medium, and 77 weak. All categories had a lower limit of 1.6Å, with upper limits of 2.8, 3.5 and 5 Å for the strong, medium and weak categories, respectively. Trial structures were generated using the simulated annealing protocol from CNS version 0.4a (anneal.inp) with SUM averaging for the nOe distances (Brunger, et al., *Acta Crystallogr. Sect. D Biol. Crystallogr.* 54:905-921 (1998); Nilges, et al., *FEBS Lett.* 239:129-136 (1988); Nilges, et al., *Protein Eng.* 2:27-38 (1988); Kuszewski, et al., *J. Magn. Reson. Ser. B* 112:79-81 (1996), Stein, et al., *J. Magn. Reson.* 124:154-164 (1997)). Twenty structures with no bond or nOe angle violations were used to represent the solution structure of MARP (see Table 1 for Root Mean Square Deviations, "RMSDs"). Structures were displayed using MOLMOL (Koradi, et al., *Science* 278:135-138 (1997)).

Example 1

Chemical synthesis and characterization of MARP

The synthesis and biochemical characterization of MARP were previously reported in Yang, et al., *supra*. The N-terminal residue (Cys-1) of MARP corresponds to the first Cys (Cys-87) of the Cys-rich region in full length, 132 residue human AGRP. The material used for the MARP NMR sample showed native-like activity as measured by its ability to competitively

inhibit NDP-MSH at MC4r, as has been shown in previous studies (Yang, et al., *supra*; Ollmann, et al., *Science* 278:135-138 (1997)). Previous mutational studies of agouti and AGRP showed residues Arg-25, Phe-26, Phe-27 (the RFF triplet, Tota, et al., *supra*) and Asp-17 (in agouti) to be determinants of receptor binding (Kiefer, et al., *Biochemistry* 36:2084-2090 (1997); Kiefer, et al., *Biochemistry* 37:991-997 (1998); Tota, et al., *supra*)).

This experiment measured inhibition of NDP-MSH stimulated cAMP generation in cells transfected with MC4r. A control experiment was conducted with no MARP present. The addition of MARP (5.0×10^{-6} M) demonstrated competitive inhibition of NDP-MSH. The addition of MARP with Arg-25 to Ala substitution (5.0×10^{-6} M) demonstrated loss of inhibition due to a mutation in the active loop. This experiment thus showed that the replacement of Arg-25 by Ala results in a complete loss of inhibitory activity.

The far-UV CD spectrum of MARP was measured in 50 mM phosphate buffer at pH 4.25 as a function of temperature with spectra shown every 5°C from 5°C to 30°C and also at 45°C. The spectrum was similar to that reported for a similar C-terminal fragment (residues 85-132) of AGRP (residues 85-132 of SEQ ID NO:1) reported by Rosenfeld, et al., *supra*, and is characterized by a negative maximum at approximately 198 nm and a slight negative maximum at 245 nm. There was little indication of canonical α-helix, however the spectrum did suggest some β character or turns. The near-UV CD spectrum, indicative of tertiary structure, showed a weak minimum at approximately 275 nm which could be attributed to the disulfide bonds and possibly restricted orientations of the side chains of the Tyr-23 and Tyr-32.

At 25°C between 20 μM and 1.0 mM there was no observable concentration dependence as measured by CD. Between 1 mM and 1.9 mM there were no concentration dependent changes in the NMR spectra (i.e. linewidths, chemical shifts, etc. all remain constant). The far-UV CD spectrum remained constant between 5°C and 45°C, a temperature range well beyond that of the present NMR experiments. Thus, by all indications, MARP existed as a monomer and did not exhibit temperature dependence under the conditions of the NMR experiments. The characteristics of the NMR spectra were indicative of a well folded protein with a single predominant conformer. $^{3}J_{HN\alpha}$ coupling constants and temperature coefficient also indicated a fully folded, non-random coil conformer as shown in Table 1 below, which shows the MARP coupling constants at 25°C and NH temperature coefficients in 50 min phosphate buffer at pH 4.2.

Table 1

Residue	$^3J_{NH\alpha}$ coupling constant (Hz)	NH temperature coefficient (ppb/°C)
Cys-1		6.93
Val-2	8.79	3.63
Arg-3	7.00	6.37
Leu-4	5.64	3.47
His-5	7.50	2.58
Glu-6	9.34	2.90
Ser-7		5.86
Cys-8	9.92	3.84
Leu-9	4.40	2.43
Gly-10		7.06
Gln-11	8.14	4.94
Gln-12	8.00	4.38
Val-13	9.03	5.56
Pro-14		0.00
Cys-15	4.33	6.74
Cys-16	5.42	3.10
Asp-17		4.81
Pro-18		0.00
Cys-19	8.81	3.84
Ala-20	9.64	3.23
Thr-21	9.36	4.04
Cys-22	5.76	7.56
Tyr-23		2.01
Cys-24		3.14
Arg-25	5.90	2.93
Phe-26	8.70	3.67
Phe-27	3.68	6.21
Asn-28	7.82	5.66
Ala-29	7.56	2.10
Phe-30	7.59	4.84
Cys-31	9.17	5.84
Tyr-32	9.49	4.43
Cys-33		1.13
Arg-34	9.34	2.74
Lys-35	7.25	8.80
Leu-36	7.74	6.43
Gly-37		7.00
Thr-38	8.07	2.07
Ala-39	4.48	7.67
Met-40	7.50	2.98
Asn-41	8.19	1.08
Pro-42		0.00
Cys-43	7.24	7.86
Ser-44	7.26	8.38

Residue	$^3J_{NH\alpha}$ coupling constant (Hz)	NH temperature coefficient (ppb/°C)
Arg-45	7.54	4.47
Thr-46		4.61

Example 2

Structural description of MARP

The minimized average NMR structure of MARP is shown in Fig. 1. Consistent with the far-UV CD spectrum, MARP showed little evidence of helix or sheet secondary structure. The disulfide bonds (1-16, 8-22, 15-33, 19-43 and 24-31) appeared to form a scaffold upon which the structure was apportioned into three major loops, which are referred to as the N-terminal loop (residues 1-18), the central loop (residues 19-34) and the C-terminal loop (residues 35-46), and are indicated in Fig. 1. RMSDs for the individual loops are reported in Table 2 below:

Table 2

Summary of MARP backbone and heavy atom RMSDs

Region (residues)	Backbone RMSD* (Å)	Heavy atom RMSD[a] (Å)
Global (1-46)	2.54	3.26
N- and active loops (1-34)	1.66	2.38
N-terminal loop (1-18)	1.31	2.03
Central loop (19-34)	1.51	2.22
Active loop (24-31)	0.69	1.53
C-terminal loop (35-46)	2.36	3.43

* Determined by fitting the family of 20 NMR structures to the minimized average structure

The N-terminal and central loops were much better defined both within the loops and with respect to each other than the C-terminal loop. The backbone RMSD for the entire protein (2.54Å) was of the same order as that of the C-terminal loop (2.36Å), while the backbone RMSD for residues 1-34 (1.66Å) was of the order of the individual N-terminal and central loops. To demonstrate limited backbone structure variability of the N-terminal and central loops, a superposition of 14 structures (selected for clarity) for residues 1-34 and the MARP minimized average structure (residues 1-46) is shown in Fig. 2.

Four of the five disulfide bonds are located at the base of the structure where they appear to pinch together the bottoms of the loops to form the "core" of the protein (Fig. 1). The exception is disulfide bond 24-31 which stabilizes the central loop. The central loop, residues

19-34, contains the RFF triplet determined to be critical for activity. This motif is situated within an even smaller, well defined loop bound by Cys-24 and Cys-31 which is referred to as the "active" loop. The side chain atoms of the RFF triplet residues are located at the surface of the protein as depicted in Fig. 3. Recent experiments further highlight the importance of this active loop. These studies demonstrate that short cyclic peptides corresponding to residues 24-31 of human AGRP do in fact antagonize MC3r and MC4r (Tota, et al., *supra*).

Inspection of the family of NMR structures and consideration of the observed HX revealed a structure for the central loop that is best described as an irregular hairpin with a well defined loop from Cys-24 to Cys-31 (RMSD 0.6Å, Fig. 3) and a stem region which is both twisted around and curved along its *z*-axis (Fig. 1). This characterization was supported by critical examination of the nOe, $^3J_{HN\alpha}$ and chemical shift data. As shown in Fig. 3, the active loop is highly constrained with the RFF triplet side chains exposed to solvent. Arg-25 and Phe-27 point out into the solvent, while one face of the Phe-26 aromatic ring rests parallel against the surface of the protein. Though the active loop satisfies several of the determinants for an Ω-loop (Leszczynski, et al., *Science* 234:849-855 (1986)), the side chain orientation of Arg-25 and Phe-27 precludes its definition as such since Ω-loop side chains generally pack within the loop of backbone atoms.

HX experiments demonstrated that the amide protons of residues Cys-8, Ala-20, Thr-1, Tyr-23, Tyr-2, Cys-3 and Arg-34 are protected from exchange with solvent. To explore whether these results were consistent with the average structure, the program DSSP (Kabsch, et al., *Biopolymers* 22:2577-2637 (1983)) was used to identify potential hydrogen bonds. DSSP identified the backbone amides of Ala-20, Thr-21, Tyr-23, and Arg-34 as potential hydrogen bond donors. In addition, solvent accessible surface area calculations showed that residues Cys-8 and Cys-33 were completely buried from solvent, though in the D_2O spectrum the αN crosspeaks of these two residues overlap, thus their individual protection from exchange is uncertain. Tyr-32 had only 8% solvent accessible surface area at the $C_\delta$protons.

The NMR structure gave a well resolved fold, however, as mentioned previously, canonical helices and β-sheets were not identified on the basis of nOes or other protocols including the chemical shift index (Wishart, et al., *Biochemistry* 31:1647-1651 (1992)) or $^3J_{HN\alpha}$ coupling constants (Wüthrich, K., *supra*).

The guidelines for these protocols assigned secondary structure on the basis of four or more consecutive residues with similar conformational shifts or $^3J_{HN\alpha}$. Helical structure was

characterized by $^3J_{HN\alpha}$<6 Hz and negative conformational shifts and β-sheet by $^3J_{HN\alpha}$>8 Hz and positive conformational shifts. Even in the active loop and stem region of the central loop, no regular secondary structure was identified by these criteria. However, the chemical shift index pointed towards a possible extended strand from residue 31 to residue 35. Table 3 sets forth the ^{1}H chemical shifts at 15°C, $^3J_{NH\alpha}$, at 250°C, and the NH temperature coefficients.

Table 3

MARP ^{1}H chemical shifts at 15°C in 50 mm phosphate buffer at pH 4.2

Residue	NH (ppm)	H_α (ppm)	H_β (ppm)	Other (ppm)
Cys- 1	8.00	5.00	3.12, 2.81	
Val-2	8.92	4.18	1.94, 2.08	CH^γ_3 0.91, 0.80
Arg-3	8.91	4.01	1.67, 1.95	H^γ1.69, 1.82, H^δ3.24, 3.25, NH 7.31
Leu-4	8.13	3.72	1.34, 1.54	H^γ1.03, CH^δ_3 0.79
His-5	8.99	4.25	3.70, 1.42	H^{δ}7.22, H^{ε}8.51
Glu-6	8.04	4.66	2.16	H^γ2.24
Ser-7	8.63	4.68	3.84	
Cys-8	8.08	4.96	3.57, 3.13	
Leu-9	7.92	4.08	1.57, 1.44	H^γ1.44, $CH^\delta_3$0.82
Gly-10	8.78	4.07, 3.69		
Gln- 11	8.06	4.24	1.95	H^γ 2.28, $H^{\varepsilon 2}$ 6.86, 7.49
Gln- 12	8.68	4.27	2.20, 1.95	H^γ 2.30, 2.34, $H^{\varepsilon 2}$ 6.82, 7.45
Val- 13	7.48	4.47	2.05	CH^γ_3 0.79, 0.90
Pro-14	8.81	4.55	2.25, 1.99	H^γ 1.84, 1.99, H^δ 3.65, 3.77
Cys-15		4.92	3.35, 1.74	
Cys-16	9.58	4.22	2.62, 3.17	
Asp-17	8.19	4.78	2.63, 2.41	
Pro-18	8.88	4.50	2.35	H^γ 1.98, H^δ 3.89, 4.04
Cys-19		4.72	2.94, 3.47	
Ala-20	8.01	4.94	1.26	
Thr-21	8.76	4.58	4.00	CH^γ_3 1.16
Cys-22	8.94	4.58	2.94, 3.02	
Tyr-23	8.71	4.62	2.79	H^δ 6.93, H^ε 6.74
Cys-24	8.30	4.91	3.23, 2.60	
Arg-25	8.29	3.84	1.83, 1.55	H^γ1.56, 1.23, H^δ 3.06
Phe-26	7.91	4.74	2.80, 3.32	H^δ 7.26, H^ε 7.42
Phe-27	8.54	4.19	3.12, 3.02	H^δ 7.17, H^ε 7.33
Asn-28	8.48	4.20	2.39, 7.77	$H^{\delta 2}$ 6.66, 7.30
Ala-29	7.75	4.38	1.25	
Phe-30	8.38	4.21	3.32, 3.36	
Cys-31	8.34	5.63	2.59, 3.03	
Tyr-32	8.88	5.20	2.59, 2.81	H^δ 6.92, H^ε 6.66
Cys-33	8.19	4.96	3.21, 2.66	
Arg-34	9.43	4.75	1.83, 1.70	H^γ1.57, 1.69, H^δ 2.62, 2.88,

Residue	NH (ppm)	H_α (ppm)	H_β (ppm)	Other (ppm)
				NH 7.08
Lys-35	9.09	4.47	1.82, 1.68	H^γ1.25, 1.43, H^δ 1.64, H^ε 2.89
Leu-36	8.77	4.3640	1.63	H^γ1.52, CH^δ_3 0.82, 0.70
Gly-37	8.48	4.09, 4.01		
Thr-38	7.82	4.42	4.42	CH^γ_3 1.21
Ala-39	8.53	4.16	1.42	
Met-40	8.06	4.40	1.95, 2.09	H^γ2.51, 2.62
Asn-41	7.74	5.00	2.62, 2.81	$H^{\delta 2}$ 7.67, 6.95
Pro-42	8.68	4.47	2.29	H^γ1.94, H^δ 3.61, 3.67
Cys-43		4.59	3.22, 3.13	
Ser-44	8.37	4.46	3.86	
Arg-45	8.26	4.43	1.93	H^γ1.66, H^δ 3.21, NH 7.25
Thr-46	7.86	4.15	4.23	$CH^\gamma_3$1.15

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

Appendix 1

Atomic Coordinates for MARP

	ATOM	1	CA	CYS	1	-6.900	2.269	-8.250	1.00	4.62
	ATOM	2	HA	CYS	1	-5.897	2.041	-7.920	1.00	4.59
5	ATOM	3	HB1	CYS	1	-7.160	4.143	-9.269	1.00	5.13
	ATOM	4	HB2	CYS	1	-6.230	4.254	-7.780	1.00	4.63
	ATOM	5	C	CYS	1	-7.900	1.623	-7.293	1.00	3.97
	ATOM	6	O	CYS	1	-9.007	1.425	-7.636	1.00	4.16
	ATOM	7	CB	CYS	1	-7.088	3.796	-8.249	1.00	4.78
10	ATOM	8	SG	CYS	1	-8.576	4.376	-7.358	1.00	5.12
	ATOM	9	N	CYS	1	-7.069	1.766	-9.642	1.00	5.38
	ATOM	10	HT1	CYS	1	-8.026	2.021	-9.958	1.00	5.84
	ATOM	11	HT2	CYS	1	-6.942	0.734	-9.624	1.00	5.52
	ATOM	12	HT3	CYS	1	-6.346	2.222	-10.234	1.00	5.55
15	ATOM	13	N	VAL	2	-7.445	1.356	-6.070	1.00	3.32
	ATOM	14	HN	VAL	2	-6.517	1.593	-5.848	1.00	3.27
	ATOM	15	CA	VAL	2	-8.315	0.801	-5.028	1.00	2.84
	ATOM	16	HA	VAL	2	-9.187	0.372	-5.505	1.00	3.11
	ATOM	17	CB	VAL	2	-7.624	-0.292	-4.151	1.00	2.58
20	ATOM	18	HB	VAL	2	-6.927	0.197	-3.490	1.00	2.54
	ATOM	19	CG1	VAL	2	-8.637	-1.028	-3.287	1.00	2.26
	ATOM	20	HG11	VAL	2	-9.533	-1.215	-3.860	1.00	2.67
	ATOM	21	HG12	VAL	2	-8.879	-0.427	-2.423	1.00	2.47
	ATOM	22	HG13	VAL	2	-8.214	-1.969	-2.962	1.00	2.24
25	ATOM	23	CG2	VAL	2	-6.851	-1.295	-5.000	1.00	3.27
	ATOM	24	HG21	VAL	2	-6.240	-1.922	-4.350	1.00	3.49
	ATOM	25	HG22	VAL	2	-6.214	-0.766	-5.692	1.00	3.65
	ATOM	26	HG23	VAL	2	-7.544	-1.914	-5.548	1.00	3.65
	ATOM	27	C	VAL	2	-8.750	1.967	-4.145	1.00	2.69
30	ATOM	28	O	VAL	2	-8.214	3.071	-4.285	1.00	3.12
	ATOM	29	N	ARG	3	-9.694	1.759	-3.238	1.00	2.64
	ATOM	30	HN	ARG	3	-10.100	0.873	-3.138	1.00	2.81
	ATOM	31	CA	ARG	3	-10.120	2.851	-2.380	1.00	2.90
	ATOM	32	HA	ARG	3	-10.214	3.738	-2.998	1.00	3.25
35	ATOM	33	CB	ARG	3	-11.466	2.539	-1.720	1.00	3.25
	ATOM	34	HB1	ARG	3	-11.575	3.157	-0.841	1.00	3.44
	ATOM	35	HB2	ARG	3	-11.477	1.501	-1.422	1.00	3.38
	ATOM	36	CG	ARG	3	-12.662	2.785	-2.628	1.00	3.73
	ATOM	37	HG1	ARG	3	-13.287	1.905	-2.628	1.00	4.00
40	ATOM	38	HG2	ARG	3	-12.307	2.976	-3.630	1.00	3.91
	ATOM	39	CD	ARG	3	-13.486	3.978	-2.164	1.00	4.23
	ATOM	40	HD1	ARG	3	-13.348	4.788	-2.865	1.00	4.60
	ATOM	41	HD2	ARG	3	-13.136	4.285	-1.189	1.00	4.30
	ATOM	42	NE	ARG	3	-14.913	3.662	-2.078	1.00	4.74
45	ATOM	43	HE	ARG	3	-15.185	2.745	-2.294	1.00	4.90
	ATOM	44	CZ	ARG	3	-15.856	4.540	-1.726	1.00	5.32
	ATOM	45	NH1	ARG	3	-15.532	5.796	-1.425	1.00	5.50
	ATOM	46	HH11	ARG	3	-14.578	6.091	-1.459	1.00	5.28

ATOM	47	HH12	ARG	3	-16.246	6.446	-1.162	1.00	6.06
ATOM	48	NH2	ARG	3	-17.129	4.160	-1.677	1.00	6.01
ATOM	49	HH21	ARG	3	-17.379	3.219	-1.903	1.00	6.19
ATOM	50	HH22	ARG	3	-17.837	4.816	-1.413	1.00	6.50
ATOM	51	C	ARG	3	-9.053	3.103	-1.329	1.00	2.75
ATOM	52	O	ARG	3	-8.519	2.163	-0.738	1.00	2.50
ATOM	53	N	LEU	4	-8.725	4.372	-1.111	1.00	3.24
ATOM	54	HN	LEU	4	-9.167	5.082	-1.615	1.00	3.68
ATOM	55	CA	LEU	4	-7.708	4.726	-0.146	1.00	3.40
ATOM	56	HA	LEU	4	-6.847	4.143	-0.371	1.00	3.11
ATOM	57	CB	LEU	4	-7.351	6.196	-0.264	1.00	4.25
ATOM	58	HB1	LEU	4	-7.379	6.479	-1.304	1.00	4.60
ATOM	59	HB2	LEU	4	-6.353	6.339	0.110	1.00	4.61
ATOM	60	CG	LEU	4	-8.285	7.097	0.500	1.00	4.65
ATOM	61	HG	LEU	4	-8.344	6.741	1.509	1.00	4.56
ATOM	62	CD1	LEU	4	-7.773	8.520	0.517	1.00	5.17
ATOM	63	HD11	LEU	4	-7.232	8.714	-0.396	1.00	5.39
ATOM	64	HD12	LEU	4	-7.113	8.647	1.363	1.00	5.60
ATOM	65	HD13	LEU	4	-8.605	9.203	0.599	1.00	5.27
ATOM	66	CD2	LEU	4	-9.659	6.991	-0.103	1.00	5.22
ATOM	67	HD21	LEU	4	-10.392	7.382	0.584	1.00	5.44
ATOM	68	HD22	LEU	4	-9.857	5.945	-0.295	1.00	5.42
ATOM	69	HD23	LEU	4	-9.690	7.541	-1.030	1.00	5.58
ATOM	70	C	LEU	4	-8.186	4.418	1.274	1.00	3.37
ATOM	71	O	LEU	4	-9.386	4.241	1.507	1.00	3.67
ATOM	72	N	HIS	5	-7.244	4.346	2.217	1.00	3.48
ATOM	73	HN	HIS	5	-6.311	4.491	1.965	1.00	3.68
ATOM	74	CA	HIS	5	-7.564	4.044	3.618	1.00	3.77
ATOM	75	HA	HIS	5	-6.629	3.939	4.150	1.00	3.80
ATOM	76	CB	HIS	5	-8.361	5.193	4.240	1.00	4.60
ATOM	77	HB1	HIS	5	-8.651	4.923	5.245	1.00	4.82
ATOM	78	HB2	HIS	5	-9.248	5.373	3.650	1.00	4.71
ATOM	79	CG	HIS	5	-7.583	6.467	4.309	1.00	5.19
ATOM	80	ND1	HIS	5	-8.108	7.657	4.768	1.00	5.95
ATOM	81	HD1	HIS	5	-9.021	7.792	5.094	1.00	6.16
ATOM	82	CD2	HIS	5	-6.300	6.730	3.966	1.00	5.27
ATOM	83	HD2	HIS	5	-5.586	6.017	3.564	1.00	4.82
ATOM	84	CE1	HIS	5	-7.182	8.597	4.702	1.00	6.46
ATOM	85	HE1	HIS	5	-7.307	9.629	4.993	1.00	7.11
ATOM	86	NE2	HIS	5	-6.076	8.060	4.221	1.00	6.10
ATOM	87	HE2	HIS	5	-5.204	8.506	4.184	1.00	6.44
ATOM	88	C	HIS	5	-8.338	2.726	3.736	1.00	3.44
ATOM	89	O	HIS	5	-8.921	2.423	4.779	1.00	3.81
ATOM	90	N	GLU	6	-8.318	1.949	2.656	1.00	2.90
ATOM	91	HN	GLU	6	-7.824	2.247	1.869	1.00	2.77
ATOM	92	CA	GLU	6	-8.984	0.664	2.592	1.00	2.75
ATOM	93	HA	GLU	6	-9.613	0.554	3.462	1.00	3.21
ATOM	94	CB	GLU	6	-9.838	0.592	1.327	1.00	2.53
ATOM	95	HB1	GLU	6	-9.174	0.488	0.478	1.00	2.28

	ATOM	96	HB2	GLU	6	-10.392	1.513	1.227	1.00	2.71
	ATOM	97	CG	GLU	6	-10.825	-0.566	1.307	1.00	2.93
	ATOM	98	HG1	GLU	6	-11.828	-0.167	1.356	1.00	3.17
	ATOM	99	HG2	GLU	6	-10.645	-1.192	2.168	1.00	3.31
	ATOM	100	CD	GLU	6	-10.701	-1.416	0.055	1.00	3.19
	ATOM	101	OE1	GLU	6	-11.236	-1.006	-0.997	1.00	3.57
	ATOM	102	OE2	GLU	6	-10.068	-2.491	0.127	1.00	3.55
	ATOM	103	C	GLU	6	-7.955	-0.456	2.557	1.00	2.53
	ATOM	104	O	GLU	6	-8.269	-1.602	2.876	1.00	2.98
	ATOM	105	N	SER	7	-6.729	-0.115	2.130	1.00	2.02
	ATOM	106	HN	SER	7	-6.557	0.801	1.870	1.00	1.87
	ATOM	107	CA	SER	7	-5.656	-1.086	2.013	1.00	1.98
	ATOM	108	HA	SER	7	-4.753	-0.563	1.693	1.00	1.74
	ATOM	109	CB	SER	7	-5.409	-1.725	3.371	1.00	2.69
	ATOM	110	HB1	SER	7	-5.970	-2.643	3.442	1.00	3.00
	ATOM	111	HB2	SER	7	-5.742	-1.040	4.133	1.00	2.94
	ATOM	112	OG	SER	7	-4.036	-2.005	3.572	1.00	3.30
	ATOM	113	HG	SER	7	-3.939	-2.883	3.947	1.00	3.67
	ATOM	114	C	SER	7	-6.066	-2.147	0.984	1.00	2.01
	ATOM	115	O	SER	7	-7.207	-2.143	0.517	1.00	2.80
	ATOM	116	N	CYS	8	-5.180	-3.074	0.643	1.00	1.78
	ATOM	117	HN	CYS	8	-4.285	-3.074	1.044	1.00	1.85
	ATOM	118	CA	CYS	8	-5.558	-4.119	-0.301	1.00	2.24
	ATOM	119	HA	CYS	8	-6.476	-3.820	-0.745	1.00	2.23
	ATOM	120	HB1	CYS	8	-3.606	-4.528	-1.025	1.00	2.70
	ATOM	121	HB2	CYS	8	-4.503	-3.311	-1.949	1.00	2.24
	ATOM	122	C	CYS	8	-5.763	-5.451	0.432	1.00	2.87
	ATOM	123	O	CYS	8	-5.575	-6.522	-0.140	1.00	3.43
	ATOM	124	CB	CYS	8	-4.568	-4.261	-1.425	1.00	2.52
	ATOM	125	SG	CYS	8	-5.047	-5.510	-2.659	1.00	3.51
	ATOM	126	N	LEU	9	-6.155	-5.353	1.714	1.00	3.11
	ATOM	127	HN	LEU	9	-6.288	-4.468	2.091	1.00	3.01
	ATOM	128	CA	LEU	9	-6.400	-6.509	2.578	1.00	3.82
	ATOM	129	HA	LEU	9	-7.159	-7.116	2.111	1.00	4.03
	ATOM	130	CB	LEU	9	-5.122	-7.346	2.756	1.00	4.45
	ATOM	131	HB1	LEU	9	-5.161	-7.820	3.726	1.00	4.87
	ATOM	132	HB2	LEU	9	-4.275	-6.676	2.739	1.00	4.44
	ATOM	133	CG	LEU	9	-4.890	-8.438	1.701	1.00	4.92
	ATOM	134	HG	LEU	9	-4.327	-8.018	0.881	1.00	4.96
	ATOM	135	CD1	LEU	9	-4.073	-9.583	2.281	1.00	5.65
	ATOM	136	HD11	LEU	9	-3.633	-9.273	3.217	1.00	5.93
	ATOM	137	HD12	LEU	9	-3.291	-9.852	1.587	1.00	5.76
	ATOM	138	HD13	LEU	9	-4.715	-10.435	2.449	1.00	6.14
	ATOM	139	CD2	LEU	9	-6.214	-8.951	1.149	1.00	5.23
	ATOM	140	HD21	LEU	9	-6.026	-9.607	0.313	1.00	5.47
	ATOM	141	HD22	LEU	9	-6.812	-8.111	0.821	1.00	5.42
	ATOM	142	HD23	LEU	9	-6.743	-9.490	1.921	1.00	5.45
	ATOM	143	C	LEU	9	-6.919	-6.051	3.948	1.00	3.94
	ATOM	144	O	LEU	9	-7.894	-6.604	4.462	1.00	4.53

ATOM	145	N	GLY	10	-6.267	-5.033	4.530	1.00	3.63
ATOM	146	HN	GLY	10	-5.501	-4.627	4.073	1.00	3.32
ATOM	147	CA	GLY	10	-6.681	-4.513	5.824	1.00	4.02
ATOM	148	HA1	GLY	10	-5.848	-4.575	6.507	1.00	4.32
ATOM	149	HA2	GLY	10	-7.490	-5.120	6.203	1.00	4.54
ATOM	150	C	GLY	10	-7.146	-3.066	5.745	1.00	3.77
ATOM	151	O	GLY	10	-8.151	-2.771	5.104	1.00	4.10
ATOM	152	N	GLN	11	-6.406	-2.169	6.402	1.00	3.61
ATOM	153	HN	GLN	11	-5.613	-2.477	6.886	1.00	3.76
ATOM	154	CA	GLN	11	-6.727	-0.736	6.407	1.00	3.67
ATOM	155	HA	GLN	11	-6.984	-0.452	5.397	1.00	3.70
ATOM	156	CB	GLN	11	-7.923	-0.451	7.325	1.00	4.51
ATOM	157	HB1	GLN	11	-7.690	0.399	7.949	1.00	4.89
ATOM	158	HB2	GLN	11	-8.094	-1.312	7.954	1.00	4.88
ATOM	159	CG	GLN	11	-9.210	-0.148	6.570	1.00	4.88
ATOM	160	HG1	GLN	11	-9.043	-0.320	5.517	1.00	5.09
ATOM	161	HG2	GLN	11	-9.467	0.889	6.727	1.00	4.85
ATOM	162	CD	GLN	11	-10.376	-1.008	7.023	1.00	5.60
ATOM	163	OE1	GLN	11	-10.540	-2.140	6.568	1.00	6.07
ATOM	164	NE2	GLN	11	-11.195	-0.474	7.924	1.00	6.05
ATOM	165	HE21	GLN	11	-11.006	0.433	8.244	1.00	5.94
ATOM	166	HE22	GLN	11	-11.958	-1.009	8.229	1.00	6.67
ATOM	167	C	GLN	11	-5.510	0.082	6.852	1.00	3.45
ATOM	168	O	GLN	11	-5.279	0.248	8.052	1.00	4.02
ATOM	169	N	GLN	12	-4.718	0.570	5.893	1.00	2.94
ATOM	170	HN	GLN	12	-4.930	0.397	4.955	1.00	2.77
ATOM	171	CA	GLN	12	-3.520	1.338	6.219	1.00	3.05
ATOM	172	HA	GLN	12	-3.399	1.297	7.292	1.00	3.57
ATOM	173	CB	GLN	12	-2.294	0.679	5.573	1.00	3.41
ATOM	174	HB1	GLN	12	-1.434	1.314	5.730	1.00	3.57
ATOM	175	HB2	GLN	12	-2.471	0.583	4.512	1.00	3.50
ATOM	176	CG	GLN	12	-1.976	-0.700	6.130	1.00	4.12
ATOM	177	HG1	GLN	12	-1.131	-1.105	5.591	1.00	4.31
ATOM	178	HG2	GLN	12	-2.834	-1.340	5.984	1.00	4.40
ATOM	179	CD	GLN	12	-1.639	-0.671	7.609	1.00	4.78
ATOM	180	OE1	GLN	12	-0.584	-0.178	8.007	1.00	5.27
ATOM	181	NE2	GLN	12	-2.538	-1.199	8.436	1.00	5.19
ATOM	182	HE21	GLN	12	-3.359	-1.576	8.052	1.00	5.11
ATOM	183	HE22	GLN	12	-2.342	-1.191	9.396	1.00	5.76
ATOM	184	C	GLN	12	-3.640	2.820	5.813	1.00	2.91
ATOM	185	O	GLN	12	-4.438	3.561	6.388	1.00	3.44
ATOM	186	N	VAL	13	-2.821	3.248	4.843	1.00	2.75
ATOM	187	HN	VAL	13	-2.199	2.625	4.444	1.00	2.86
ATOM	188	CA	VAL	13	-2.795	4.629	4.377	1.00	3.05
ATOM	189	HA	VAL	13	-3.226	5.246	5.153	1.00	3.57
ATOM	190	CB	VAL	13	-1.331	5.091	4.154	1.00	3.46
ATOM	191	HB	VAL	13	-1.291	6.137	4.363	1.00	3.85
ATOM	192	CG1	VAL	13	-0.380	4.402	5.127	1.00	3.80
ATOM	193	HG11	VAL	13	-0.395	3.337	4.956	1.00	3.78

ATOM	194	HG12	VAL	13	-0.691	4.607	6.140	1.00	4.15
ATOM	195	HG13	VAL	13	0.622	4.776	4.976	1.00	4.28
ATOM	196	CG2	VAL	13	-0.878	4.877	2.709	1.00	3.89
ATOM	197	HG21	VAL	13	-0.918	5.815	2.176	1.00	4.21
ATOM	198	HG22	VAL	13	-1.528	4.163	2.224	1.00	4.18
ATOM	199	HG23	VAL	13	0.135	4.503	2.700	1.00	4.14
ATOM	200	C	VAL	13	-3.615	4.813	3.090	1.00	3.04
ATOM	201	O	VAL	13	-4.211	3.857	2.589	1.00	2.95
ATOM	202	N	PRO	14	-3.671	6.053	2.539	1.00	3.66
ATOM	203	CA	PRO	14	-4.438	6.341	1.318	1.00	4.09
ATOM	204	HA	PRO	14	-5.448	5.975	1.401	1.00	4.39
ATOM	205	CB	PRO	14	-4.454	7.870	1.263	1.00	5.04
ATOM	206	HB1	PRO	14	-5.342	8.242	1.750	1.00	5.62
ATOM	207	HB2	PRO	14	-4.437	8.197	0.234	1.00	5.18
ATOM	208	CG	PRO	14	-3.224	8.289	1.986	1.00	5.32
ATOM	209	HG1	PRO	14	-3.368	9.268	2.418	1.00	5.91
ATOM	210	HG2	PRO	14	-2.384	8.296	1.308	1.00	5.77
ATOM	211	CD	PRO	14	-3.010	7.267	3.068	1.00	4.46
ATOM	212	HD1	PRO	14	-3.474	7.590	3.989	1.00	4.68
ATOM	213	HD2	PRO	14	-1.956	7.102	3.216	1.00	4.53
ATOM	214	C	PRO	14	-3.795	5.765	0.053	1.00	3.77
ATOM	215	O	PRO	14	-2.821	5.013	0.129	1.00	4.07
ATOM	216	N	CYS	15	-4.341	6.138	-1.114	1.00	3.60
ATOM	217	HN	CYS	15	-5.104	6.751	-1.110	1.00	3.75
ATOM	218	CA	CYS	15	-3.814	5.676	-2.394	1.00	3.70
ATOM	219	HA	CYS	15	-2.780	5.986	-2.427	1.00	3.90
ATOM	220	HB1	CYS	15	-4.871	3.825	-2.642	1.00	4.62
ATOM	221	HB2	CYS	15	-3.464	3.720	-1.587	1.00	4.34
ATOM	222	C	CYS	15	-4.533	6.333	-3.587	1.00	3.85
ATOM	223	O	CYS	15	-4.584	7.561	-3.661	1.00	4.33
ATOM	224	CB	CYS	15	-3.851	4.149	-2.500	1.00	4.05
ATOM	225	SG	CYS	15	-2.856	3.502	-3.887	1.00	4.02
ATOM	226	N	CYS	16	-5.057	5.523	-4.536	1.00	3.70
ATOM	227	HN	CYS	16	-4.985	4.556	-4.441	1.00	3.50
ATOM	228	CA	CYS	16	-5.710	6.043	-5.736	1.00	4.08
ATOM	229	HA	CYS	16	-5.636	5.275	-6.496	1.00	3.93
ATOM	230	HB1	CYS	16	-7.294	7.293	-4.999	1.00	4.46
ATOM	231	HB2	CYS	16	-7.597	5.564	-4.810	1.00	4.09
ATOM	232	C	CYS	16	-4.979	7.301	-6.249	1.00	4.59
ATOM	233	O	CYS	16	-5.610	8.273	-6.672	1.00	5.05
ATOM	234	CB	CYS	16	-7.196	6.327	-5.471	1.00	4.36
ATOM	235	SG	CYS	16	-8.216	6.341	-6.984	1.00	5.13
ATOM	236	N	ASP	17	-3.630	7.259	-6.197	1.00	4.60
ATOM	237	HN	ASP	17	-3.198	6.451	-5.847	1.00	4.30
ATOM	238	CA	ASP	17	-2.777	8.374	-6.639	1.00	5.13
ATOM	239	HA	ASP	17	-2.901	8.469	-7.708	1.00	5.59
ATOM	240	CB	ASP	17	-3.219	9.692	-5.975	1.00	5.62
ATOM	241	HB1	ASP	17	-3.503	9.496	-4.953	1.00	5.76
ATOM	242	HB2	ASP	17	-4.072	10.083	-6.508	1.00	5.74

ATOM	243	CG	ASP	17	-2.132	10.754	-5.977	1.00	6.17
ATOM	244	OD1	ASP	17	-1.748	11.210	-7.075	1.00	6.53
ATOM	245	OD2	ASP	17	-1.668	11.129	-4.880	1.00	6.56
ATOM	246	C	ASP	17	-1.283	8.093	-6.351	1.00	4.80
ATOM	247	O	ASP	17	-0.465	8.102	-7.271	1.00	4.56
ATOM	248	N	PRO	18	-0.908	7.839	-5.070	1.00	5.13
ATOM	249	CA	PRO	18	0.485	7.553	-4.669	1.00	5.06
ATOM	250	HA	PRO	18	1.165	8.301	-5.023	1.00	5.33
ATOM	251	CB	PRO	18	0.438	7.600	-3.127	1.00	5.85
ATOM	252	HB1	PRO	18	1.243	8.221	-2.761	1.00	6.32
ATOM	253	HB2	PRO	18	0.542	6.601	-2.729	1.00	5.79
ATOM	254	CG	PRO	18	-0.891	8.182	-2.786	1.00	6.39
ATOM	255	HG1	PRO	18	-0.814	9.257	-2.712	1.00	6.94
ATOM	256	HG2	PRO	18	-1.249	7.765	-1.857	1.00	6.75
ATOM	257	CD	PRO	18	-1.800	7.803	-3.912	1.00	5.92
ATOM	258	HD1	PRO	18	-2.598	8.519	-4.012	1.00	6.35
ATOM	259	HD2	PRO	18	-2.194	6.810	-3.762	1.00	5.99
ATOM	260	C	PRO	18	0.965	6.192	-5.162	1.00	4.35
ATOM	261	O	PRO	18	0.484	5.690	-6.181	1.00	4.45
ATOM	262	N	CYS	19	1.908	5.589	-4.438	1.00	4.00
ATOM	263	HN	CYS	19	2.257	6.027	-3.634	1.00	4.34
ATOM	264	CA	CYS	19	2.427	4.285	-4.822	1.00	3.50
ATOM	265	HA	CYS	19	2.416	4.237	-5.903	1.00	3.76
ATOM	266	HB1	CYS	19	4.354	5.069	-4.280	1.00	4.02
ATOM	267	HB2	CYS	19	4.412	3.492	-5.049	1.00	4.20
ATOM	268	C	CYS	19	1.501	3.201	-4.285	1.00	2.98
ATOM	269	O	CYS	19	0.949	2.444	-5.067	1.00	2.87
ATOM	270	CB	CYS	19	3.880	4.100	-4.333	1.00	3.68
ATOM	271	SG	CYS	19	4.070	3.305	-2.701	1.00	3.27
ATOM	272	N	ALA	20	1.301	3.192	-2.958	1.00	3.00
ATOM	273	HN	ALA	20	1.746	3.891	-2.427	1.00	3.43
ATOM	274	CA	ALA	20	0.381	2.259	-2.261	1.00	2.68
ATOM	275	HA	ALA	20	0.867	1.965	-1.352	1.00	2.43
ATOM	276	CB	ALA	20	-0.896	2.998	-1.882	1.00	2.88
ATOM	277	HB1	ALA	20	-1.747	2.351	-2.033	1.00	3.03
ATOM	278	HB2	ALA	20	-0.997	3.878	-2.499	1.00	3.21
ATOM	279	HB3	ALA	20	-0.847	3.293	-0.841	1.00	3.09
ATOM	280	C	ALA	20	0.077	0.972	-3.049	1.00	2.88
ATOM	281	O	ALA	20	-0.665	1.040	-3.996	1.00	3.63
ATOM	282	N	THR	21	0.639	-0.179	-2.606	1.00	2.52
ATOM	283	HN	THR	21	1.178	-0.127	-1.824	1.00	2.17
ATOM	284	CA	THR	21	0.492	-1.519	-3.247	1.00	3.01
ATOM	285	HA	THR	21	0.640	-1.411	-4.288	1.00	3.44
ATOM	286	CB	THR	21	1.615	-2.430	-2.712	1.00	3.51
ATOM	287	HB	THR	21	2.292	-1.827	-2.145	1.00	3.87
ATOM	288	OG1	THR	21	2.342	-3.018	-3.765	1.00	3.92
ATOM	289	HG1	THR	21	1.829	-3.731	-4.147	1.00	4.04
ATOM	290	CG2	THR	21	1.139	-3.534	-1.814	1.00	3.64
ATOM	291	HG21	THR	21	1.148	-4.469	-2.352	1.00	3.73

ATOM	292	HG22	THR	21	0.138	-3.302	-1.513	1.00	3.73
ATOM	293	HG23	THR	21	1.776	-3.606	-0.946	1.00	3.90
ATOM	294	C	THR	21	-0.893	-2.141	-3.040	1.00	2.82
ATOM	295	O	THR	21	-1.823	-1.429	-2.757	1.00	2.79
ATOM	296	N	CYS	22	-1.047	-3.453	-3.225	1.00	3.23
ATOM	297	HN	CYS	22	-0.284	-3.987	-3.488	1.00	3.75
ATOM	298	CA	CYS	22	-2.361	-4.091	-3.092	1.00	3.29
ATOM	299	HA	CYS	22	-2.941	-3.546	-2.350	1.00	2.73
ATOM	300	HB1	CYS	22	-2.576	-4.621	-5.160	1.00	4.32
ATOM	301	HB2	CYS	22	-3.066	-2.975	-4.783	1.00	4.25
ATOM	302	C	CYS	22	-2.268	-5.563	-2.656	1.00	3.83
ATOM	303	O	CYS	22	-2.573	-6.463	-3.444	1.00	4.63
ATOM	304	CB	CYS	22	-3.092	-3.999	-4.442	1.00	3.86
ATOM	305	SG	CYS	22	-4.841	-4.522	-4.418	1.00	3.79
ATOM	306	N	TYR	23	-1.888	-5.785	-1.377	1.00	3.64
ATOM	307	HN	TYR	23	-1.689	-5.024	-0.821	1.00	3.23
ATOM	308	CA	TYR	23	-1.799	-7.133	-0.783	1.00	4.31
ATOM	309	HA	TYR	23	-2.770	-7.368	-0.375	1.00	4.75
ATOM	310	CB	TYR	23	-1.483	-8.137	-1.875	1.00	4.99
ATOM	311	HB1	TYR	23	-0.990	-7.599	-2.663	1.00	4.83
ATOM	312	HB2	TYR	23	-2.406	-8.540	-2.250	1.00	5.71
ATOM	313	CG	TYR	23	-0.599	-9.294	-1.468	1.00	5.19
ATOM	314	CD1	TYR	23	-1.141	-10.531	-1.148	1.00	5.34
ATOM	315	HD1	TYR	23	-2.212	-10.660	-1.183	1.00	5.46
ATOM	316	CD2	TYR	23	0.776	-9.147	-1.418	1.00	5.53
ATOM	317	HD2	TYR	23	1.205	-8.183	-1.658	1.00	5.77
ATOM	318	CE1	TYR	23	-0.332	-11.592	-0.790	1.00	5.56
ATOM	319	HE1	TYR	23	-0.771	-12.547	-0.545	1.00	5.82
ATOM	320	CE2	TYR	23	1.595	-10.200	-1.059	1.00	5.81
ATOM	321	HE2	TYR	23	2.667	-10.065	-1.025	1.00	6.26
ATOM	322	CZ	TYR	23	1.036	-11.421	-0.748	1.00	5.71
ATOM	323	OH	TYR	23	1.846	-12.475	-0.395	1.00	6.01
ATOM	324	HH	TYR	23	2.160	-12.917	-1.187	1.00	6.05
ATOM	325	C	TYR	23	-0.764	-7.219	0.355	1.00	4.08
ATOM	326	O	TYR	23	-0.942	-7.990	1.300	1.00	4.31
ATOM	327	N	CYS	24	0.305	-6.427	0.252	1.00	3.99
ATOM	328	HN	CYS	24	0.376	-5.836	-0.520	1.00	4.20
ATOM	329	CA	CYS	24	1.379	-6.392	1.250	1.00	3.97
ATOM	330	HA	CYS	24	2.008	-5.546	0.990	1.00	3.54
ATOM	331	HB1	CYS	24	0.849	-7.090	3.203	1.00	4.45
ATOM	332	HB2	CYS	24	-0.159	-5.779	2.608	1.00	4.72
ATOM	333	C	CYS	24	2.230	-7.664	1.271	1.00	4.56
ATOM	334	O	CYS	24	1.702	-8.775	1.300	1.00	4.95
ATOM	335	CB	CYS	24	0.841	-6.167	2.655	1.00	4.21
ATOM	336	SG	CYS	24	1.818	-4.991	3.578	1.00	3.97
ATOM	337	N	ARG	25	3.553	-7.485	1.306	1.00	4.81
ATOM	338	HN	ARG	25	3.910	-6.572	1.315	1.00	4.70
ATOM	339	CA	ARG	25	4.485	-8.614	1.370	1.00	5.41
ATOM	340	HA	ARG	25	4.148	-9.364	0.676	1.00	5.78

	ATOM	341	CB	ARG	25	5.903	-8.174	0.986	1.00	5.35
	ATOM	342	HB1	ARG	25	6.586	-8.982	1.206	1.00	5.48
	ATOM	343	HB2	ARG	25	6.173	-7.317	1.584	1.00	5.55
	ATOM	344	CG	ARG	25	6.065	-7.802	-0.478	1.00	5.19
5	ATOM	345	HG1	ARG	25	5.093	-7.787	-0.945	1.00	5.37
	ATOM	346	HG2	ARG	25	6.687	-8.541	-0.961	1.00	5.36
	ATOM	347	CD	ARG	25	6.712	-6.433	-0.636	1.00	5.12
	ATOM	348	HD1	ARG	25	6.606	-5.890	0.292	1.00	5.27
	ATOM	349	HD2	ARG	25	6.201	-5.899	-1.423	1.00	4.97
10	ATOM	350	NE	ARG	25	8.134	-6.527	-0.973	1.00	5.60
	ATOM	351	HE	ARG	25	8.367	-6.596	-1.923	1.00	5.89
	ATOM	352	CZ	ARG	25	9.125	-6.525	-0.074	1.00	5.97
	ATOM	353	NH1	ARG	25	8.863	-6.428	1.227	1.00	5.99
	ATOM	354	HH11	ARG	25	7.920	-6.356	1.546	1.00	5.75
15	ATOM	355	HH12	ARG	25	9.614	-6.428	1.888	1.00	6.45
	ATOM	356	NH2	ARG	25	10.386	-6.622	-0.477	1.00	6.62
	ATOM	357	HH21	ARG	25	10.595	-6.697	-1.451	1.00	6.89
	ATOM	358	HH22	ARG	25	11.127	-6.620	0.194	1.00	6.99
	ATOM	359	C	ARG	25	4.514	-9.217	2.773	1.00	5.75
20	ATOM	360	O	ARG	25	4.289	-10.415	2.952	1.00	6.36
	ATOM	361	N	PHE	26	4.802	-8.369	3.759	1.00	5.44
	ATOM	362	HN	PHE	26	4.967	-7.433	3.534	1.00	4.97
	ATOM	363	CA	PHE	26	4.879	-8.793	5.158	1.00	5.87
	ATOM	364	HA	PHE	26	4.990	-9.862	5.151	1.00	6.37
25	ATOM	365	CB	PHE	26	6.121	-8.187	5.838	1.00	5.87
	ATOM	366	HB1	PHE	26	6.993	-8.742	5.519	1.00	6.14
	ATOM	367	HB2	PHE	26	6.026	-8.279	6.907	1.00	5.89
	ATOM	368	CG	PHE	26	6.361	-6.740	5.515	1.00	5.68
	ATOM	369	CD1	PHE	26	6.033	-5.748	6.420	1.00	5.91
30	ATOM	370	HD1	PHE	26	5.595	-6.022	7.368	1.00	6.30
	ATOM	371	CD2	PHE	26	6.920	-6.381	4.303	1.00	5.59
	ATOM	372	HD2	PHE	26	7.172	-7.156	3.593	1.00	5.76
	ATOM	373	CE1	PHE	26	6.259	-4.419	6.122	1.00	5.89
	ATOM	374	HE1	PHE	26	5.999	-3.653	6.837	1.00	6.28
35	ATOM	375	CE2	PHE	26	7.150	-5.054	3.995	1.00	5.57
	ATOM	376	HE2	PHE	26	7.587	-4.787	3.044	1.00	5.74
	ATOM	377	CZ	PHE	26	6.819	-4.071	4.907	1.00	5.65
	ATOM	378	HZ	PHE	26	6.997	-3.032	4.670	1.00	5.75
	ATOM	379	C	PHE	26	3.576	-8.452	5.906	1.00	5.78
40	ATOM	380	O	PHE	26	2.491	-8.694	5.373	1.00	5.69
	ATOM	381	N	PHE	27	3.669	-7.902	7.131	1.00	5.97
	ATOM	382	HN	PHE	27	4.549	-7.733	7.517	1.00	6.18
	ATOM	383	CA	PHE	27	2.477	-7.552	7.910	1.00	6.04
	ATOM	384	HA	PHE	27	1.921	-8.463	8.076	1.00	6.43
45	ATOM	385	CB	PHE	27	2.876	-6.962	9.273	1.00	6.49
	ATOM	386	HB1	PHE	27	2.391	-6.006	9.402	1.00	6.27
	ATOM	387	HB2	PHE	27	3.945	-6.822	9.299	1.00	6.51
	ATOM	388	CG	PHE	27	2.497	-7.831	10.440	1.00	7.32
	ATOM	389	CD1	PHE	27	3.134	-9.042	10.657	1.00	7.85

ATOM	390	HD1	PHE	27	3.910	-9.363	9.977	1.00	7.76
ATOM	391	CD2	PHE	27	1.503	-7.433	11.321	1.00	7.78
ATOM	392	HD2	PHE	27	1.000	-6.491	11.161	1.00	7.65
ATOM	393	CE1	PHE	27	2.787	-9.840	11.731	1.00	8.65
ATOM	394	HE1	PHE	27	3.290	-10.783	11.889	1.00	9.17
ATOM	395	CE2	PHE	27	1.151	-8.227	12.395	1.00	8.58
ATOM	396	HE2	PHE	27	0.375	-7.905	13.074	1.00	9.03
ATOM	397	CZ	PHE	27	1.794	-9.432	12.601	1.00	8.94
ATOM	398	HZ	PHE	27	1.521	-10.054	13.440	1.00	9.59
ATOM	399	C	PHE	27	1.587	-6.578	7.130	1.00	5.30
ATOM	400	O	PHE	27	0.738	-7.010	6.348	1.00	5.60
ATOM	401	N	ASN	28	1.790	-5.270	7.320	1.00	4.68
ATOM	402	HN	ASN	28	2.489	-4.973	7.933	1.00	4.87
ATOM	403	CA	ASN	28	1.011	-4.272	6.600	1.00	3.97
ATOM	404	HA	ASN	28	0.924	-4.630	5.591	1.00	3.66
ATOM	405	CB	ASN	28	-0.399	-4.127	7.180	1.00	4.05
ATOM	406	HB1	ASN	28	-0.734	-3.109	7.042	1.00	4.27
ATOM	407	HB2	ASN	28	-0.372	-4.353	8.235	1.00	4.30
ATOM	408	CG	ASN	28	-1.411	-5.060	6.513	1.00	4.05
ATOM	409	OD1	ASN	28	-2.267	-5.631	7.187	1.00	4.69
ATOM	410	ND2	ASN	28	-1.320	-5.227	5.185	1.00	3.69
ATOM	411	HD21	ASN	28	-0.620	-4.749	4.698	1.00	3.34
ATOM	412	HD22	ASN	28	-1.961	-5.829	4.749	1.00	4.01
ATOM	413	C	ASN	28	1.730	-2.924	6.548	1.00	3.77
ATOM	414	O	ASN	28	1.560	-2.065	7.416	1.00	3.98
ATOM	415	N	ALA	29	2.500	-2.756	5.480	1.00	3.54
ATOM	416	HN	ALA	29	2.541	-3.464	4.828	1.00	3.55
ATOM	417	CA	ALA	29	3.237	-1.556	5.187	1.00	3.45
ATOM	418	HA	ALA	29	2.593	-0.713	5.396	1.00	3.44
ATOM	419	CB	ALA	29	4.495	-1.408	5.996	1.00	3.85
ATOM	420	HB1	ALA	29	4.945	-0.462	5.737	1.00	4.00
ATOM	421	HB2	ALA	29	5.173	-2.212	5.761	1.00	3.97
ATOM	422	HB3	ALA	29	4.255	-1.422	7.047	1.00	4.27
ATOM	423	C	ALA	29	3.563	-1.564	3.712	1.00	3.33
ATOM	424	O	ALA	29	4.201	-2.484	3.193	1.00	4.12
ATOM	425	N	PHE	30	3.068	-0.553	3.063	1.00	2.67
ATOM	426	HN	PHE	30	2.558	0.083	3.580	1.00	2.45
ATOM	427	CA	PHE	30	3.209	-0.378	1.613	1.00	2.74
ATOM	428	HA	PHE	30	2.894	0.626	1.371	1.00	2.83
ATOM	429	CB	PHE	30	4.669	-0.566	1.171	1.00	3.35
ATOM	430	HB1	PHE	30	4.796	-0.143	0.185	1.00	3.67
ATOM	431	HB2	PHE	30	4.891	-1.622	1.133	1.00	3.59
ATOM	432	CG	PHE	30	5.673	0.083	2.088	1.00	3.86
ATOM	433	CD1	PHE	30	5.441	1.346	2.615	1.00	4.35
ATOM	434	HD1	PHE	30	4.529	1.867	2.361	1.00	4.45
ATOM	435	CD2	PHE	30	6.847	-0.573	2.424	1.00	4.38
ATOM	436	HD2	PHE	30	7.040	-1.557	2.020	1.00	4.49
ATOM	437	CE1	PHE	30	6.360	1.938	3.459	1.00	5.11
ATOM	438	HE1	PHE	30	6.167	2.922	3.862	1.00	5.69

	ATOM	439	CE2	PHE	30	7.769	0.016	3.266	1.00	5.15
	ATOM	440	HE2	PHE	30	8.681	-0.505	3.520	1.00	5.77
	ATOM	441	CZ	PHE	30	7.525	1.273	3.785	1.00	5.42
	ATOM	442	HZ	PHE	30	8.245	1.735	4.444	1.00	6.14
	ATOM	443	C	PHE	30	2.287	-1.369	0.894	1.00	2.53
	ATOM	444	O	PHE	30	2.704	-2.085	-0.011	1.00	3.13
	ATOM	445	N	CYS	31	1.034	-1.406	1.362	1.00	2.06
	ATOM	446	HN	CYS	31	0.818	-0.818	2.101	1.00	2.12
	ATOM	447	CA	CYS	31	-0.017	-2.306	0.854	1.00	1.97
	ATOM	448	HA	CYS	31	0.224	-2.580	-0.153	1.00	2.16
	ATOM	449	HB1	CYS	31	0.505	-4.333	1.205	1.00	2.78
	ATOM	450	HB2	CYS	31	-1.059	-3.866	1.863	1.00	2.85
	ATOM	451	C	CYS	31	-1.410	-1.651	0.917	1.00	1.56
	ATOM	452	O	CYS	31	-1.944	-1.466	2.011	1.00	2.04
	ATOM	453	CB	CYS	31	-0.037	-3.553	1.710	1.00	2.51
	ATOM	454	SG	CYS	31	0.725	-3.308	3.341	1.00	2.99
	ATOM	455	N	TYR	32	-1.997	-1.284	-0.236	1.00	1.35
	ATOM	456	HN	TYR	32	-1.539	-1.446	-1.075	1.00	1.77
	ATOM	457	CA	TYR	32	-3.327	-0.635	-0.238	1.00	1.25
	ATOM	458	HA	TYR	32	-3.903	-1.138	0.503	1.00	1.48
	ATOM	459	CB	TYR	32	-3.228	0.842	0.167	1.00	1.68
	ATOM	460	HB1	TYR	32	-4.003	1.065	0.885	1.00	1.99
	ATOM	461	HB2	TYR	32	-3.365	1.461	-0.707	1.00	2.16
	ATOM	462	CG	TYR	32	-1.909	1.201	0.781	1.00	2.00
	ATOM	463	CD1	TYR	32	-0.740	1.028	0.066	1.00	2.67
	ATOM	464	HD1	TYR	32	-0.802	0.652	-0.942	1.00	3.10
	ATOM	465	CD2	TYR	32	-1.829	1.678	2.069	1.00	2.45
	ATOM	466	HD2	TYR	32	-2.743	1.831	2.638	1.00	2.72
	ATOM	467	CE1	TYR	32	0.484	1.317	0.605	1.00	3.29
	ATOM	468	HE1	TYR	32	1.382	1.166	0.001	1.00	3.99
	ATOM	469	CE2	TYR	32	-0.612	1.976	2.629	1.00	3.14
	ATOM	470	HE2	TYR	32	-0.576	2.335	3.635	1.00	3.79
	ATOM	471	CZ	TYR	32	0.550	1.794	1.900	1.00	3.39
	ATOM	472	OH	TYR	32	1.768	2.097	2.465	1.00	4.22
	ATOM	473	HH	TYR	32	1.978	3.019	2.301	1.00	4.50
	ATOM	474	C	TYR	32	-4.105	-0.726	-1.553	1.00	1.55
	ATOM	475	O	TYR	32	-5.142	-1.384	-1.621	1.00	2.20
	ATOM	476	N	CYS	33	-3.660	0.019	-2.571	1.00	2.04
	ATOM	477	HN	CYS	33	-2.872	0.584	-2.442	1.00	2.31
	ATOM	478	CA	CYS	33	-4.394	0.077	-3.831	1.00	2.83
	ATOM	479	HA	CYS	33	-5.173	-0.658	-3.776	1.00	3.09
	ATOM	480	HB1	CYS	33	-5.053	1.928	-2.929	1.00	3.26
	ATOM	481	HB2	CYS	33	-6.070	1.345	-4.241	1.00	3.20
	ATOM	482	C	CYS	33	-3.578	-0.177	-5.137	1.00	3.55
	ATOM	483	O	CYS	33	-4.167	-0.120	-6.219	1.00	4.43
	ATOM	484	CB	CYS	33	-5.055	1.457	-3.915	1.00	3.01
	ATOM	485	SG	CYS	33	-4.215	2.580	-5.069	1.00	3.40
	ATOM	486	N	ARG	34	-2.255	-0.404	-5.084	1.00	3.34
	ATOM	487	HN	ARG	34	-1.777	-0.399	-4.203	1.00	2.80

	ATOM	488	CA	ARG	34	-1.467	-0.586	-6.333	1.00	4.11
	ATOM	489	HA	ARG	34	-2.019	-0.101	-7.125	1.00	4.69
	ATOM	490	CB	ARG	34	-0.137	0.169	-6.148	1.00	4.21
	ATOM	491	HB1	ARG	34	0.270	-0.122	-5.193	1.00	4.15
5	ATOM	492	HB2	ARG	34	-0.360	1.220	-6.109	1.00	4.16
	ATOM	493	CG	ARG	34	0.964	-0.004	-7.166	1.00	4.98
	ATOM	494	HG1	ARG	34	0.969	0.843	-7.835	1.00	5.57
	ATOM	495	HG2	ARG	34	0.809	-0.914	-7.721	1.00	5.02
	ATOM	496	CD	ARG	34	2.301	-0.067	-6.425	1.00	5.32
10	ATOM	497	HD1	ARG	34	2.654	-1.087	-6.429	1.00	5.23
	ATOM	498	HD2	ARG	34	2.143	0.253	-5.385	1.00	5.43
	ATOM	499	NE	ARG	34	3.314	0.793	-7.034	1.00	6.08
	ATOM	500	HE	ARG	34	3.149	1.112	-7.946	1.00	6.18
	ATOM	501	CZ	ARG	34	4.441	1.162	-6.420	1.00	6.88
15	ATOM	502	NH1	ARG	34	4.701	0.747	-5.183	1.00	7.12
	ATOM	503	HH11	ARG	34	4.052	0.155	-4.705	1.00	6.71
	ATOM	504	HH12	ARG	34	5.546	1.028	-4.728	1.00	7.87
	ATOM	505	NH2	ARG	34	5.313	1.944	-7.048	1.00	7.69
	ATOM	506	HH21	ARG	34	5.125	2.257	-7.979	1.00	7.76
20	ATOM	507	HH22	ARG	34	6.157	2.222	-6.589	1.00	8.36
	ATOM	508	C	ARG	34	-1.287	-2.074	-6.731	1.00	4.36
	ATOM	509	O	ARG	34	-2.259	-2.732	-7.106	1.00	4.73
	ATOM	510	N	LYS	35	-0.057	-2.590	-6.668	1.00	4.53
	ATOM	511	HN	LYS	35	0.674	-2.028	-6.364	1.00	4.61
25	ATOM	512	CA	LYS	35	0.255	-3.975	-7.032	1.00	4.93
	ATOM	513	HA	LYS	35	-0.509	-4.613	-6.607	1.00	5.03
	ATOM	514	CB	LYS	35	0.293	-4.170	-8.558	1.00	5.89
	ATOM	515	HB1	LYS	35	-0.714	-4.346	-8.907	1.00	6.19
	ATOM	516	HB2	LYS	35	0.895	-5.039	-8.780	1.00	6.25
30	ATOM	517	CG	LYS	35	0.863	-2.989	-9.331	1.00	6.31
	ATOM	518	HG1	LYS	35	0.552	-2.075	-8.852	1.00	6.46
	ATOM	519	HG2	LYS	35	0.479	-3.014	-10.339	1.00	6.32
	ATOM	520	CD	LYS	35	2.384	-3.025	-9.387	1.00	6.93
	ATOM	521	HD1	LYS	35	2.689	-3.525	-10.295	1.00	7.08
35	ATOM	522	HD2	LYS	35	2.755	-3.570	-8.532	1.00	7.20
	ATOM	523	CE	LYS	35	2.972	-1.623	-9.373	1.00	7.41
	ATOM	524	HE1	LYS	35	3.647	-1.538	-8.533	1.00	7.65
	ATOM	525	HE2	LYS	35	2.169	-0.911	-9.260	1.00	7.56
	ATOM	526	NZ	LYS	35	3.716	-1.314	-10.626	1.00	7.75
40	ATOM	527	HZ1	LYS	35	3.082	-1.396	-11.447	1.00	7.81
	ATOM	528	HZ2	LYS	35	4.509	-1.975	-10.746	1.00	8.01
	ATOM	529	HZ3	LYS	35	4.091	-0.344	-10.588	1.00	7.95
	ATOM	530	C	LYS	35	1.596	-4.335	-6.418	1.00	4.53
	ATOM	531	O	LYS	35	2.432	-3.453	-6.214	1.00	4.71
45	ATOM	532	N	LEU	36	1.805	-5.602	-6.086	1.00	4.30
	ATOM	533	HN	LEU	36	1.130	-6.257	-6.243	1.00	4.47
	ATOM	534	CA	LEU	36	3.036	-5.989	-5.453	1.00	4.13
	ATOM	535	HA	LEU	36	3.354	-5.105	-4.916	1.00	3.77
	ATOM	536	CB	LEU	36	2.843	-7.055	-4.416	1.00	4.20

ATOM	537	HB1	LEU	36	3.786	-7.540	-4.232	1.00	4.50
ATOM	538	HB2	LEU	36	2.120	-7.776	-4.770	1.00	4.66
ATOM	539	CG	LEU	36	2.360	-6.422	-3.135	1.00	3.78
ATOM	540	HG	LEU	36	2.622	-5.367	-3.189	1.00	3.57
ATOM	541	CD1	LEU	36	0.849	-6.524	-3.055	1.00	4.07
ATOM	542	HD11	LEU	36	0.535	-6.468	-2.028	1.00	4.35
ATOM	543	HD12	LEU	36	0.528	-7.464	-3.481	1.00	4.26
ATOM	544	HD13	LEU	36	0.405	-5.712	-3.610	1.00	4.30
ATOM	545	CD2	LEU	36	3.040	-7.016	-1.907	1.00	4.09
ATOM	546	HD21	LEU	36	4.020	-6.576	-1.793	1.00	4.26
ATOM	547	HD22	LEU	36	3.138	-8.084	-2.027	1.00	4.48
ATOM	548	HD23	LEU	36	2.447	-6.802	-1.026	1.00	4.28
ATOM	549	C	LEU	36	4.137	-6.293	-6.405	1.00	4.76
ATOM	550	O	LEU	36	4.441	-7.429	-6.775	1.00	5.31
ATOM	551	N	GLY	37	4.752	-5.198	-6.707	1.00	4.97
ATOM	552	HN	GLY	37	4.412	-4.392	-6.286	1.00	4.82
ATOM	553	CA	GLY	37	5.915	-5.144	-7.545	1.00	5.70
ATOM	554	HA1	GLY	37	5.776	-4.401	-8.313	1.00	6.04
ATOM	555	HA2	GLY	37	6.065	-6.105	-7.993	1.00	6.19
ATOM	556	C	GLY	37	7.120	-4.776	-6.704	1.00	5.54
ATOM	557	O	GLY	37	7.963	-3.978	-7.115	1.00	6.08
ATOM	558	N	THR	38	7.161	-5.356	-5.495	1.00	5.00
ATOM	559	HN	THR	38	6.446	-5.959	-5.236	1.00	4.79
ATOM	560	CA	THR	38	8.202	-5.119	-4.526	1.00	4.96
ATOM	561	HA	THR	38	8.007	-5.786	-3.699	1.00	5.16
ATOM	562	CB	THR	38	9.591	-5.445	-5.075	1.00	5.78
ATOM	563	HB	THR	38	10.154	-4.531	-5.184	1.00	6.05
ATOM	564	OG1	THR	38	9.526	-6.095	-6.337	1.00	6.18
ATOM	565	HG1	THR	38	9.079	-6.941	-6.244	1.00	6.46
ATOM	566	CG2	THR	38	10.345	-6.343	-4.136	1.00	6.38
ATOM	567	HG21	THR	38	9.756	-7.232	-3.961	1.00	6.46
ATOM	568	HG22	THR	38	10.500	-5.827	-3.199	1.00	6.55
ATOM	569	HG23	THR	38	11.296	-6.614	-4.570	1.00	6.89
ATOM	570	C	THR	38	8.149	-3.700	-3.987	1.00	4.17
ATOM	571	O	THR	38	8.747	-2.781	-4.553	1.00	4.31
ATOM	572	N	ALA	39	7.460	-3.542	-2.852	1.00	3.68
ATOM	573	HN	ALA	39	7.044	-4.326	-2.437	1.00	3.92
ATOM	574	CA	ALA	39	7.370	-2.242	-2.190	1.00	3.19
ATOM	575	HA	ALA	39	6.953	-1.533	-2.893	1.00	3.39
ATOM	576	CB	ALA	39	6.459	-2.305	-0.952	1.00	2.97
ATOM	577	HB1	ALA	39	5.453	-1.989	-1.214	1.00	3.17
ATOM	578	HB2	ALA	39	6.845	-1.652	-0.184	1.00	3.05
ATOM	579	HB3	ALA	39	6.429	-3.317	-0.578	1.00	3.21
ATOM	580	C	ALA	39	8.789	-1.809	-1.820	1.00	3.22
ATOM	581	O	ALA	39	9.116	-0.625	-1.846	1.00	3.39
ATOM	582	N	MET	40	9.628	-2.825	-1.539	1.00	3.43
ATOM	583	HN	MET	40	9.270	-3.743	-1.595	1.00	3.65
ATOM	584	CA	MET	40	11.056	-2.668	-1.215	1.00	3.68
ATOM	585	HA	MET	40	11.340	-3.533	-0.635	1.00	3.98

ATOM	586	CB	MET	40	11.888	-2.677	-2.505	1.00	4.33
ATOM	587	HB1	MET	40	12.568	-1.837	-2.491	1.00	4.41
ATOM	588	HB2	MET	40	11.222	-2.575	-3.350	1.00	4.46
ATOM	589	CG	MET	40	12.704	-3.945	-2.692	1.00	5.16
ATOM	590	HG1	MET	40	12.841	-4.118	-3.751	1.00	5.45
ATOM	591	HG2	MET	40	12.162	-4.774	-2.262	1.00	5.31
ATOM	592	SD	MET	40	14.325	-3.847	-1.907	1.00	6.00
ATOM	593	CE	MET	40	15.309	-3.173	-3.244	1.00	6.60
ATOM	594	HE1	MET	40	15.124	-3.736	-4.146	1.00	6.92
ATOM	595	HE2	MET	40	16.356	-3.239	-2.987	1.00	6.56
ATOM	596	HE3	MET	40	15.041	-2.139	-3.404	1.00	7.01
ATOM	597	C	MET	40	11.386	-1.423	-0.381	1.00	3.37
ATOM	598	O	MET	40	11.559	-1.513	0.835	1.00	3.68
ATOM	599	N	ASN	41	11.510	-0.272	-1.048	1.00	3.35
ATOM	600	HN	ASN	41	11.386	-0.269	-2.021	1.00	3.59
ATOM	601	CA	ASN	41	11.864	0.977	-0.377	1.00	3.57
ATOM	602	HA	ASN	41	12.586	0.745	0.390	1.00	4.02
ATOM	603	CB	ASN	41	12.503	1.948	-1.377	1.00	4.36
ATOM	604	HB1	ASN	41	12.506	2.941	-0.952	1.00	4.61
ATOM	605	HB2	ASN	41	11.920	1.953	-2.286	1.00	4.37
ATOM	606	CG	ASN	41	13.932	1.569	-1.727	1.00	5.30
ATOM	607	OD1	ASN	41	14.191	0.473	-2.226	1.00	5.84
ATOM	608	ND2	ASN	41	14.868	2.477	-1.470	1.00	5.81
ATOM	609	HD21	ASN	41	14.592	3.331	-1.074	1.00	5.65
ATOM	610	HD22	ASN	41	15.798	2.256	-1.685	1.00	6.50
ATOM	611	C	ASN	41	10.651	1.636	0.277	1.00	3.02
ATOM	612	O	ASN	41	9.507	1.390	-0.108	1.00	3.02
ATOM	613	N	PRO	42	10.898	2.490	1.291	1.00	3.12
ATOM	614	CA	PRO	42	9.833	3.192	2.018	1.00	3.15
ATOM	615	HA	PRO	42	9.154	2.496	2.484	1.00	3.54
ATOM	616	CB	PRO	42	10.588	3.974	3.102	1.00	4.06
ATOM	617	HB1	PRO	42	10.553	3.428	4.033	1.00	4.67
ATOM	618	HB2	PRO	42	10.133	4.945	3.230	1.00	4.13
ATOM	619	CG	PRO	42	11.983	4.089	2.593	1.00	4.46
ATOM	620	HG1	PRO	42	12.673	4.160	3.421	1.00	5.17
ATOM	621	HG2	PRO	42	12.072	4.956	1.954	1.00	4.81
ATOM	622	CD	PRO	42	12.235	2.834	1.810	1.00	3.83
ATOM	623	HD1	PRO	42	12.611	2.055	2.456	1.00	4.33
ATOM	624	HD2	PRO	42	12.927	3.025	1.002	1.00	3.84
ATOM	625	C	PRO	42	9.043	4.145	1.125	1.00	2.55
ATOM	626	O	PRO	42	9.610	5.065	0.531	1.00	2.68
ATOM	627	N	CYS	43	7.731	3.923	1.041	1.00	2.61
ATOM	628	HN	CYS	43	7.340	3.178	1.541	1.00	3.08
ATOM	629	CA	CYS	43	6.862	4.768	0.230	1.00	2.66
ATOM	630	HA	CYS	43	7.382	5.025	-0.623	1.00	3.10
ATOM	631	HB1	CYS	43	4.942	3.935	0.709	1.00	2.52
ATOM	632	HB2	CYS	43	5.840	3.031	-0.503	1.00	2.27
ATOM	633	C	CYS	43	6.535	6.042	0.962	1.00	3.31
ATOM	634	O	CYS	43	6.796	7.152	0.494	1.00	3.75

	ATOM	635	CB	CYS	43	5.579	4.021	-0.159	1.00	2.41
	ATOM	636	SG	CYS	43	4.616	4.831	-1.478	1.00	3.14
	ATOM	637	N	SER	44	5.994	5.829	2.121	1.00	3.89
	ATOM	638	HN	SER	44	5.876	4.902	2.362	1.00	3.89
5	ATOM	639	CA	SER	44	5.605	6.891	3.055	1.00	4.87
	ATOM	640	HA	SER	44	4.903	6.474	3.757	1.00	5.17
	ATOM	641	CB	SER	44	6.842	7.366	3.832	1.00	5.33
	ATOM	642	HB1	SER	44	7.327	6.513	4.284	1.00	5.78
	ATOM	643	HB2	SER	44	6.539	8.054	4.606	1.00	5.38
10	ATOM	644	OG	SER	44	7.771	8.016	2.981	1.00	5.71
	ATOM	645	HG	SER	44	8.433	7.384	2.689	1.00	5.91
	ATOM	646	C	SER	44	4.916	8.060	2.338	1.00	5.60
	ATOM	647	O	SER	44	4.324	7.887	1.270	1.00	5.82
	ATOM	648	N	ARG	45	4.992	9.244	2.946	1.00	6.31
15	ATOM	649	HN	ARG	45	5.466	9.302	3.798	1.00	6.38
	ATOM	650	CA	ARG	45	4.385	10.456	2.392	1.00	7.28
	ATOM	651	HA	ARG	45	4.713	11.288	2.997	1.00	7.53
	ATOM	652	CB	ARG	45	4.845	10.690	0.944	1.00	7.96
	ATOM	653	HB1	ARG	45	4.706	11.733	0.701	1.00	8.31
20	ATOM	654	HB2	ARG	45	4.231	10.094	0.285	1.00	7.94
	ATOM	655	CG	ARG	45	6.301	10.331	0.684	1.00	8.60
	ATOM	656	HG1	ARG	45	6.471	10.321	-0.383	1.00	8.78
	ATOM	657	HG2	ARG	45	6.498	9.351	1.089	1.00	8.86
	ATOM	658	CD	ARG	45	7.254	11.328	1.326	1.00	9.04
25	ATOM	659	HD1	ARG	45	6.712	11.904	2.062	1.00	9.20
	ATOM	660	HD2	ARG	45	7.632	11.990	0.560	1.00	8.91
	ATOM	661	NE	ARG	45	8.384	10.664	1.981	1.00	9.74
	ATOM	662	HE	ARG	45	8.404	10.675	2.960	1.00	9.93
	ATOM	663	CZ	ARG	45	9.375	10.047	1.328	1.00	10.31
30	ATOM	664	NH1	ARG	45	9.388	10.005	-0.002	1.00	10.31
	ATOM	665	HH11	ARG	45	8.653	10.435	-0.525	1.00	9.86
	ATOM	666	HH12	ARG	45	10.134	9.541	-0.479	1.00	10.88
	ATOM	667	NH2	ARG	45	10.357	9.466	2.011	1.00	11.06
	ATOM	668	HH21	ARG	45	10.355	9.490	3.011	1.00	11.21
35	ATOM	669	HH22	ARG	45	11.100	9.004	1.525	1.00	11.58
	ATOM	670	C	ARG	45	2.858	10.387	2.457	1.00	7.69
	ATOM	671	O	ARG	45	2.168	10.651	1.468	1.00	8.34
	ATOM	672	N	THR	46	2.336	10.036	3.633	1.00	7.60
	ATOM	673	HN	THR	46	2.935	9.842	4.379	1.00	7.26
40	ATOM	674	CA	THR	46	0.895	9.934	3.836	1.00	8.32
	ATOM	675	HA	THR	46	0.488	9.396	2.997	1.00	8.41
	ATOM	676	CB	THR	46	0.582	9.157	5.122	1.00	8.61
	ATOM	677	HB	THR	46	-0.480	9.219	5.314	1.00	8.70
	ATOM	678	OG1	THR	46	1.266	9.717	6.233	1.00	8.93
45	ATOM	679	HG1	THR	46	2.175	9.410	6.243	1.00	9.05
	ATOM	680	CG2	THR	46	0.951	7.688	5.037	1.00	8.89
	ATOM	681	HG21	THR	46	0.433	7.232	4.206	1.00	9.07
	ATOM	682	HG22	THR	46	0.668	7.191	5.954	1.00	9.06
	ATOM	683	HG23	THR	46	2.017	7.593	4.891	1.00	9.01

ATOM	684	C	THR	46	0.249	11.319	3.889	1.00	9.05
ATOM	685	OT1	THR	46	0.759	12.187	4.632	1.00	9.34
ATOM	686	OT2	THR	46	-0.761	11.525	3.184	1.00	9.53

END

CLAIMS

What is claimed is:

1. A method of identifying a compound that modulates ligand binding to a melanocortin receptor, said method comprising: modeling test compounds that fit spatially into a melanocortin receptor ligand binding site of interest using an atomic structural model of a melanocortin receptor binding region or portion thereof; screening said test compounds in an assay characterized by binding of a test compound to a melanocortin receptor ligand binding site; and identifying a test compound that modulates ligand binding to said melanocortin receptor.

2. The method of Claim 1 wherein said melanocortin receptor binding region comprises the minimized agouti related protein receptor binding region or portion thereof.

3. The method of Claim 1 wherein said atomic structural model comprises atomic coordinates of amino acid residues corresponding to residues 1-18 of the N-terminal loop of the minimized agouti related protein (residues 1-18 of SEQ ID NO:2), residues 19-13 of the central loop of the minimized agouti related protein (residues 19-34 of SEQ ID NO:2), and residues 35-46 of the C-terminal loop of the minimized agouti related protein (residues 35-46 of SEQ ID NO:2).

4. The method of Claim 1 wherein said atomic structural model comprises atomic coordinates of amino acid residues corresponding to residues 19-34 of the central loop of the minimized agouti related protein (residues 19-34 of SEQ ID NO:2) and at least residues 15-18 of the N-terminal loop of the minimized agouti related protein (residues 15-18 of SEQ ID NO:2).

5. The method of Claim 1 wherein said atomic structural model comprises atomic coordinates of amino acid residues corresponding to residues 19-34 of the central loop of the minimized agouti related protein (residues 19-34 of SEQ ID NO:2) and at least 20% of the contiguous or non-contiguous residues or their atoms are selected from residues 1-18 of the N-terminal loop of the minimized agouti related protein (residues 1-18 of SEQ ID NO:2).

6. The method of Claim 1 wherein said atomic structural model comprises atomic coordinates of amino acid residues corresponding to residues 24-31 of the active loop of the minimized agouti related protein (residues 24-31 of SEQ ID NO:2).

7. The method of Claim 1 wherein said atomic structural model comprises atomic coordinates of amino acid residues corresponding to residues 25-27 of the active loop of the minimized agouti related protein (residues 25-27 of SEQ ID NO:2).

8. The method of Claim 1 wherein said screening is *in vitro*.

9. The method of Claim 8 wherein said screening is high throughput screening.

10. The method of Claim 1 wherein said assay is a biological assay.

11. The method of Claim 1 wherein said test compound is from a library of compounds.

12. The method of Claim 1 wherein said test compound is an agonist or antagonist of ligand binding.

13. The method of Claim 12 wherein said test compound is a small organic molecule, a peptide, or peptidomimetic.

14. A method for identifying an agonist or antagonist of ligand binding to a melanocortin receptor, said method comprising the steps of: providing the atomic coördinates of a melanocortin receptor binding region or portion thereof to a computerized modeling system; modeling compounds which match or mimic the receptor binding region and thus fit spatially into the melanocortin receptor ligand binding site; and identifying in an assay for melanocortin receptor activity a compound that increases or decreases the activity of said melanocortin receptor by binding the ligand binding site of said melanocortin receptor, whereby an agonist or antagonist of ligand binding is identified.

15. The method of Claim 14 wherein said melanocortin receptor binding region comprises the minimized agouti related protein receptor binding region or portion thereof.

16. A machine-readable data storage medium, comprising a data storage material encoded with machine readable data which, when using a machine programmed with instructions for using said data, is capable of displaying a graphical three-dimensional representation of a molecule that binds a melanocortin receptor comprising structure coordinates of amino acid residues corresponding to residues 1-18 of the N-terminal loop of the minimized agouti related protein (residues 1-18 of SEQ ID NO:2), residues 19-13 of the central loop of the minimized agouti related protein (residues 19-34 of SEQ ID NO:2), and residues 35-46 of the C-terminal loop of the minimized agouti related protein (residues 35-46 of SEQ ID NO:2), or a homologue of said molecule.

17. The machine readable storage medium of Claim 16 wherein said molecule is a melanocortin receptor agonist.

18. The machine readable storage medium of Claim 16 wherein said molecule is a melanocortin receptor antagonist.

19. The machine-readable data storage medium according to Claim 16 wherein said molecule is defined by the set of structure coordinates depicted in Appendix 1, or a homologue of said molecule, said homologue having a root mean square deviation from the backbone atoms of said amino acids of not more than 2.54Å.

20. A machine-readable data storage medium comprising a data storage material encoded with a first set of machine readable data which, when combined with a second set of machine readable data, using a machine programmed with instructions for using said first set of data and said second set of data, can determine at least a portion of the structure coordinates corresponding to the second set of machine readable data, wherein: said first set of data comprises a Fourier transform of at least a portion of the structural coordinates selected from the group consisting of coordinates depicted in Appendix 1; and said second set of data comprises an X-ray diffraction pattern of a molecule.

21. An NMR structure of the minimized agouti related protein, embodied in a computer readable media.

22. A compound identified according to the method of Claim 1.

23. A polypeptide comprising the amino acid sequence:

CVRLHESCLGQQVPCCDPAATCYCRFFNAFCYC (SEQ ID NO:3)

or a modified form thereof.

24. The polypeptide of Claim 23 wherein the polypeptide is chemically synthesized.

25. A method of treating a disease state in mammals that is alleviated by treatment with a polypeptide having an amino acid sequence:

CVRLHESCLGQQVPCCDPAATCYCRFFNAFCYC (SEQ ID NO:3)

which method comprises administering to a mammal in need of such a treatment a therapeutically effective amount of said polypeptide, or a pharmaceutically acceptable salt thereof.

26. The method of Claim 25wherein said disease state is a wasting syndrome.

27. A pharmaceutical composition comprising a therapeutically effective amount of a polypeptide of the sequence:

CVRLHESCLGQQVPCCDPAATCYCRFFNAFCYC (SEQ ID NO:3)

or a pharmaceutically acceptable salt thereof.

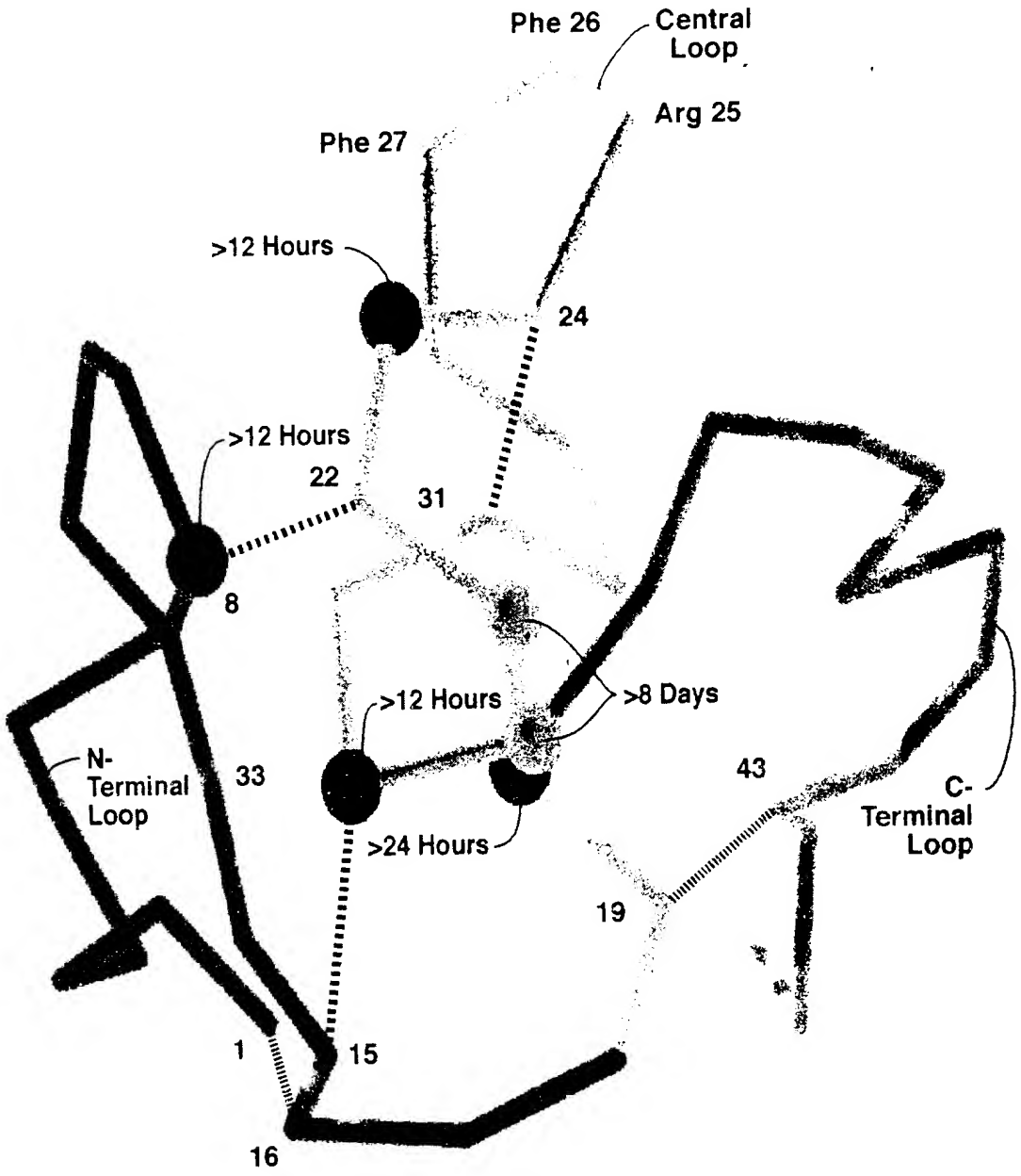
Phe 26
Central Loop
Arg 25
Phe 27
>12 Hours
24
>12 Hours
22
31
8
>12 Hours
>8 Days
N-Terminal Loop
33
43
C-Terminal Loop
>24 Hours
19
1
15
16

FIG._1

FIG._2

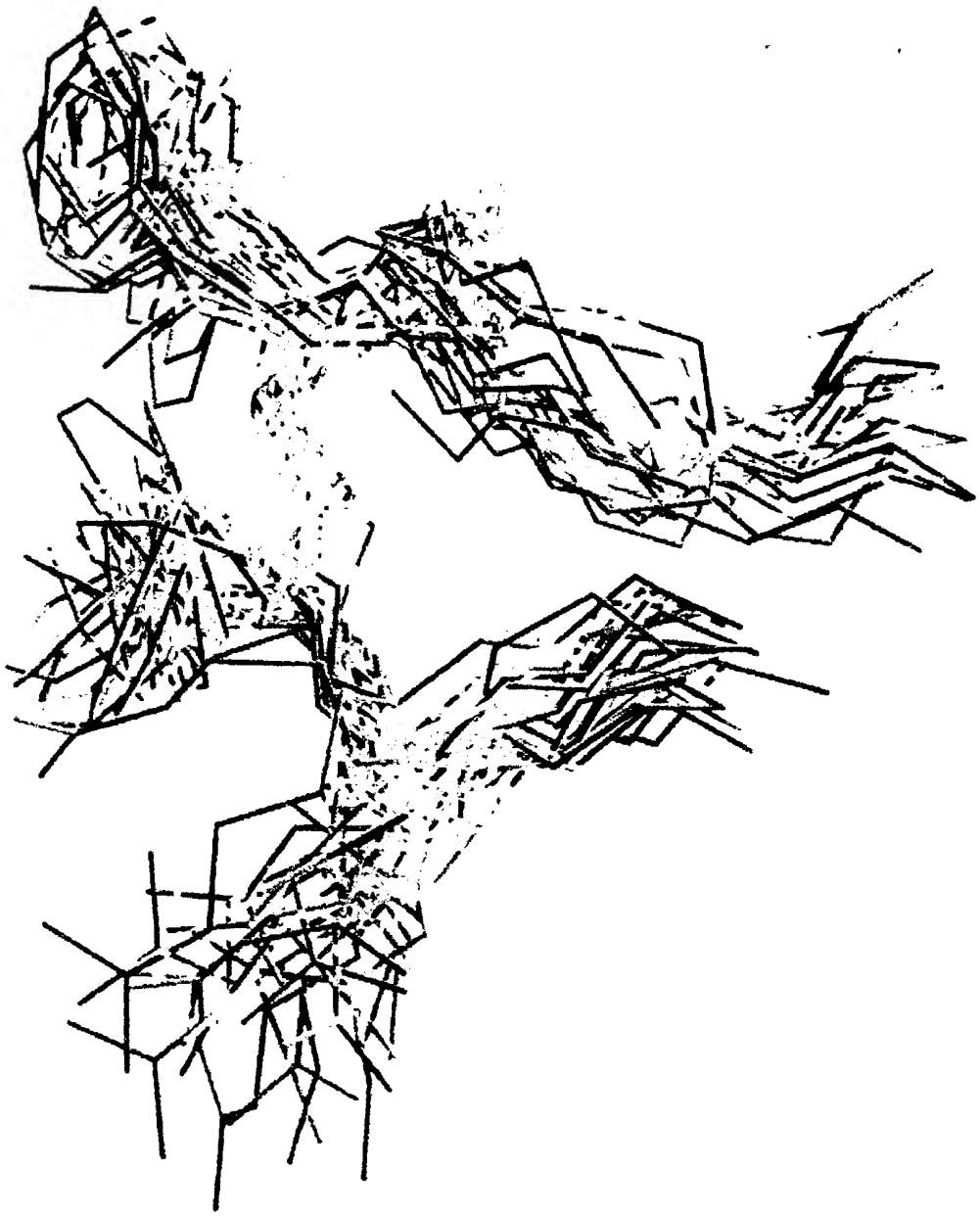

FIG._3

SEQUENCE LISTING

<110> The Regents of the University of California

<120> METHODS AND COMPOUNDS FOR MODULATING MELANOCORTIN RECEPTOR-LIGAND BINDING

<130> UCAL-257/00WO

<140>
<141>

<160> 3

<170> PatentIn Ver. 2.0

<210> 1
<211> 132
<212> PRT
<213> Homo sapiens

<400> 1

Met Leu Thr Ala Ala Leu Leu Ser Cys Ala Leu Leu Leu Ala Leu Pro
1 5 10 15

Ala Thr Arg Gly Ala Gln Met Gly Leu Ala Pro Met Glu Gly Ile Arg
20 25 30

Arg Pro Asp Gln Ala Leu Leu Pro Glu Leu Pro Gly Leu Gly Leu Arg
35 40 45

Ala Pro Leu Lys Lys Thr Thr Ala Glu Gln Ala Glu Glu Asp Leu Leu
50 55 60

Gln Glu Ala Gln Ala Leu Ala Glu Val Leu Asp Leu Gln Asp Arg Glu
65 70 75 80

Pro Arg Ser Ser Arg Arg Cys Val Arg Leu His Glu Ser Cys Leu Gly
85 90 95

Gln Gln Val Pro Cys Cys Asp Pro Cys Ala Thr Cys Tyr Cys Arg Phe
100 105 110

Phe Asn Ala Phe Cys Tyr Cys Arg Lys Leu Gly Thr Ala Met Asn Pro
115 120 125

Cys Ser Arg Thr
130

<210> 2
<211> 46
<212> PRT
<213> Homo sapiens

<400> 2
Cys Val Arg Leu His Glu Ser Cys Leu Gly Gln Gln Val Pro Cys Cys
1 5 10 15

Asp Pro Cys Ala Thr Cys Tyr Cys Arg Phe Phe Asn Ala Phe Cys Tyr
20 25 30

Cys Arg Lys Leu Gly Thr Ala Met Asn Pro Cys Ser Arg Thr
35 40 45

<210> 3
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

<400> 3
Cys Val Arg Leu His Glu Ser Cys Leu Gly Gln Gln Val Pro Cys Cys
1 5 10 15

Asp Pro Ala Ala Thr Cys Tyr Cys Arg Phe Phe Asn Ala Phe Cys Tyr
20 25 30

Cys

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/25201

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 7/00
US CL : 530/317

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/317; 530/312; 930/270

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
The American Chemical Society Journals

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS-ONLINE: STN-CAPLUS-USPATFULL; MEDLINE and The American Chemical Society Online Journals.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TOTA et al. Molecular Interaction of Agouti Protein and Agouti-Related Protein with Human Melanocortin Receptors. Biochemistry. 1999, Vol. 38, No. 3, pages 897-904, entire document.	1-22
Y	SCHIOTH et al. Thyrotropin Releasing Hormone (TRH) Selectively Binds and Activates the Melanocortin Receptor. Peptides. 1999, Vol. 20, pages 395-400, entite document.	1-22
Y	HASKELL-LUEVANO et al. Compounds that Activate the Mouse Melanocortin-1 Receptor Identified by Screening a Small Molecule Library Based on the beta-Turn. J. Med. Chem. 1999, Vol. 42, No. 21, pages 4380-4387, entire document.	13-15
Y	WO 99/50295 A2 (GRYPHON SCIENCES) 07 October 1999 (07.10.1999) pages 9-18.	23-27
Y	WO 98/27113 A2 (RUDOLPH MAGNUS INSTITUTE FOR NEUROSCIENCES) 25 June 1998 (25.06.1998) pages 5-10.	23-27
Y	WO 98/10068 A2 (OREGON HEALTH SCIENCES UNIVERSITY) 12 March 1998 (12.03.1998) pages 33-39.	23-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2000 (28.02.2000)	21 MAR 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Sherif A Mafafi, PhD Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)